2012

Effects of climate change on reptiles with temperature-dependent sex determination and potential adaptation via maternal nest-site choice

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by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Ecology and Evolutionary Biology

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Ames, Iowa

2012

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

A growing literature demonstrates numerous effects of climate change on natural systems including range shifts (Parmesan et al. 1999), changes in phenology (Crick et al. 1997), altered species interactions (Hersteinsson and Macdonald 1992), and shifts in community structure (Holbrook et al. 1997). Many of these changes are difficult to predict because they involve indirect effects of environmental changes on a multitude of organisms, and occur via complex pathways. However, in some cases, thermally-sensitive traits exist which are directly impacted by climate and have demographic consequences for populations as a whole. Temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos, is such a trait. TSD is common among reptile groups, and occurs in all crocodilians and tuatara, most turtles, and some lizards (Janzen and Paukstis 1991). The temperature range within which the complement of offspring sex shifts from all of one sex to all of the other is generally very narrow, and is often less than 1^{o}C (Ewert et al. 1994). As a consequence, population sex ratios are extraordinarily sensitive to temperature changes because a small shift in environmental temperature could dramatically alter the sex ratio of the offspring produced. Anthropogenically-caused climate change, therefore, has the potential to severely impact TSD reptiles by resulting in populations made up of predominantly one sex (Janzen 1994, Nelson et al. 2004, Mitchell et al. 2008).

Reptiles with TSD have survived periods of past climate change, suggesting some capacity to compensate for potential skews in sex ratios. Two potential mechanisms have

been proposed to explain the continued persistence of TSD reptile populations despite numerous periods of past global warming and cooling (Bulmer and Bull 1983): 1) changes in the specific temperatures producing each sex; and 2) maternal nest-site choice, which affects the microclimate experienced by developing embryos. While both mechanisms are likely to operate over evolutionary timescales, low heritability suggests that shifts in thermal sensitivity are unlikely to compensate for changes in sex ratios resulting from rapid climate change (Janzen 1994). Therefore, if TSD reptiles are to avoid skewed sex ratios produced by rising global temperatures, nest-site choice is the likely mechanism (Morjan 2003).

Dissertation organization

In this dissertation, I explore the extent to which nest-site choice may be a mechanism allowing TSD reptiles to compensate for skews in sex ratios produced by increasing climatic temperatures. Chapter 2 is a literature review exploring the major hypotheses for the evolution of nest-site choice across oviparous animals. This was the first comprehensive review of nest-site choice to be conducted for all major oviparous taxa. J.M.R. is the primary author; F.J.J. had the original idea for the review and provided feedback on the manuscript. In subsequent chapters, I use a model TSD reptile species, *Chrysemys picta*, to explore how different components of nest-site choice differ across a geographic range and the extent to which these components might compensate for the effects of climate change via behavioral phenotypic plasticity.

In Chapter 3, I describe a common-garden experiment using turtles from four transplanted and one local population to assess whether variation among populations in nest-site choice is primarily genetic or environmental. For this chapter, J.M.R. collected all field

data, conducted analyses, and wrote the manuscript; F.J.J. determined sex ratios, helped with analyses, and reviewed the manuscript. Chapter 4 describes the effects of maternal nest-site choice from the common-garden experiment in Chapter 3 on the performance of offspring produced by females from each population. The common-garden experiment provides a basis for the following chapters, which focus on several individual aspects of maternal nest-site choice.

Following the results of the common-garden experiment that choice of shade cover over nest sites is a behaviorally plastic character, Chapter 5 explores female selection of shade cover over potential nest sites. This experiment compares both shade cover selection and the relationship between shade cover and incubation regime between two populations. J.M.R. collected data on shade cover availability at the Illinois site and all data at the New Mexico site, analyzed the data, and wrote the manuscript. D.A.W. and F.J.J. collected data on shade cover use at the Illinois site and provided feedback on the manuscript.

Chapters 6 and 7 examine nest depth as a component of nest-site choice. In Chapter 6, I explore whether nest depth in turtles is constrained by female size, and therefore may not be a viable mechanism for compensating for potential changes in incubation regimes. Finally, in Chapter 7, I conduct a manipulative experiment of nest depth to determine the extent to which adjustment in nest depth changes incubation regime. J.M.R. conducted the field experiment, analyzed all data, and wrote the manuscript; B.L.B. and J.L.R. conducted hatchling performance tests; and F.J.J. collected the long-term dataset at the Illinois site and provided feedback on the manuscript.

In combination, this dissertation provides a comprehensive test of the extent to which maternal nest-site choice behavior may allow reptiles with TSD to compensate for the potential skews in sex ratios caused by climate change.

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CHAPTER 2. PUTTING EGGS IN ONE BASKET: ECOLOGICAL AND EVOLUTIONARY HYPOTHESES FOR VARIATION IN OVIPOSITION-SITE CHOICE

A paper published in *Annual Review of Ecology, Evolution and Systematics*
2010 Volume 41 pages 39-57

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Abstract

Oviposition-site choice is a major maternal effect by which females can affect the survival and phenotype of their offspring. Across oviparous species, the ultimate reasons for females' selection of oviposition sites often differ. We present six hypotheses that have been used to explain non-random oviposition-site choice in insects, fish, amphibians, reptiles, and birds: 1) maximizing embryo survival; 2) maximizing maternal survival; 3) modifying offspring phenotype; 4) proximity to suitable habitat for offspring; 5) maintaining natal philopatry; and 6) indirect oviposition-site choice via mate-choice. Because these hypotheses differ in their relevance across oviparous taxa, each hypothesis must be tested to ensure accurate understanding of the ultimate reason behind oviposition-site choice in a particular taxon. By presenting the major hypotheses for oviposition-site choice as they relate to diverse oviparous animals, we nonetheless illustrate particular trends across animal taxa while highlighting avenues for future research into the ecological and evolutionary drivers of oviposition-site choice.

Introduction

Females can influence offspring survival and phenotype through both genetic and non-genetic means. In practice, maternal effects are considered to be mechanisms that influence offspring phenotype but which, for the most part, are not transmitted genetically from mother to offspring. One such maternal effect that can have a large impact on offspring survival and phenotype is maternal choice of oviposition site (Bernardo 1996). In oviparous species, oviposition-site choice is a female's assessment of potential nest sites and selection of a particular site. A female's decision about where to lay her eggs can have serious consequences for her own reproductive fitness, as oviposition site affects embryo survival, juvenile performance, and offspring phenotype, as well as potentially the survival of the ovipositing female. Thus, oviposition-site choice is a life-history trait of critical importance (Resetarits 1996).

Scope of review

In this review, we describe six major ecological and evolutionary hypotheses purported to explain non-random oviposition-site choice in oviparous animals: 1) maximizing embryo survival; 2) maximizing maternal survival; 3) modifying offspring phenotype; 4) proximity to suitable habitat for offspring; 5) maintaining natal philopatry; and 6) indirect oviposition-site choice via mate-choice. We discuss each hypothesis as it relates to insects, fish, amphibians, non-avian reptiles (hereafter reptiles), and birds. Importantly, we examine **ultimate** ecological or evolutionary reasons for oviposition-site choice: that is, what is the fitness consequence to an individual that selects an oviposition site based on one of the above hypotheses? We do not discuss **proximate** reasons for oviposition-site choice;

namely, the environmental or social cues that an individual may use as indicators of a site's future incubation conditions.

Because the six hypotheses for oviposition-site choice presented here differ in their relevance across oviparous taxa, one cannot simply generalize the importance of any particular hypothesis from one group to others; instead, the various hypotheses must be tested for the species of interest to ensure accurate understanding of the situational mechanism(s) for oviposition-site choice operating in that species. Moreover, these hypotheses for oviposition-site choice are not mutually exclusive: females may "take into account" more than one hypothesis when making oviposition decisions, and it is likely that females in some taxa "adopt" a hierarchical approach by first choosing a general area for oviposition based on one hypothesis, and then selecting a particular site within that area based on a different hypothesis. By presenting the major hypotheses for oviposition-site choice as they relate to diverse oviparous animals, we nonetheless illustrate particular trends across various animal taxa while highlighting avenues for future research into mechanisms controlling oviposition-site choice, especially in the current context of rapid environmental change.

Terminology

We will use the general term **oviposition-site choice** or **oviposition-site selection** in this review to refer to any oviparous animal's selection of a site for deposition of eggs. However, this terminology also encompasses several more specific terms used when referring to certain taxa: **nest-site choice** refers to animals that construct a nest in which they oviposit, such as reptiles and birds, and **spawning-site choice** refers to animals with external

fertilization in which pairs copulate and oviposit simultaneously, such as some fishes and anuran amphibians.

Several hypotheses discussed here can be differentiated based on the developmental stage with which they are concerned. Hypothesis 1 deals with embryo survival, which is equivalent to the **egg stage** for all taxa discussed in this review. Hypothesis 4, in contrast, discusses the performance and survival of juveniles. In the case of indirect developers such as insects, fish, and most amphibians, the term "juvenile" is equivalent to the **larval stage** (i.e., caterpillars, fry, or tadpoles, respectively). The juvenile stage of interest for direct developers is simply hatchlings (in reptiles) or nestlings/fledglings (in birds). Finally, Hypothesis 3 discusses offspring phenotype. This hypothesis applies equally to any recently-hatched offspring, regardless of whether the taxon of interest undergoes direct or indirect development.

Hypothesis 1: Maximizing embryo survival

Maximization of embryo survival has traditionally been considered the most important reason for non-random choice of oviposition site in oviparous animals. Females can choose oviposition sites that will minimize predation risk, maintain a microclimate suitable for embryo development, or avoid habitats prone to desiccation in aquatic species. However, recent research on insects has shown that females do not necessarily oviposit in the habitat types that result in the greatest embryo survival (see Hypothesis 2), suggesting that alternative selection pressures may override differences in embryo survival among habitat types. Importantly, this result indicates that oviposition-site choice is more complex than simply choosing the location with the highest probability of embryonic survival. Therefore,

maximizing embryo survival may not govern oviposition-site choice in some and perhaps many oviparous taxa.

In insects, maximizing survival of eggs tends to be accomplished by selecting sites that minimize predation or conspecific competition. Both terrestrially- (Higashiura 1989) and aquatically-ovipositing species (Petranka & Fakhoury 1991) preferred predator-free oviposition sites. Egg survivorship decreased as more eggs were deposited on a host plant (Mitchell 1975) and, in some cases, earlier-hatching larvae fed on the eggs of conspecifics on the same plant (Williams & Gilbert 1981). Therefore, females in many species avoid ovipositing on plants that already contain eggs of conspecifics.

Oviposition-site choice in taxa with external fertilization, including many fishes and amphibians, is more complicated. The site must first allow for successful fertilization of the eggs, as well as meet the requirements of spawning adults and developing eggs at the site of oviposition. In fishes, spawning may occur in specific microhabitats that enhance fertilization success (Petersen et al. 1992). The provision of a suitable environment for the developing embryos, however, appears to be the strongest driver of oviposition-site choice in fish.

The wide diversity of habitats occupied by fishes (e.g., salt- and freshwater, fast-flowing rivers and calm pools, and climates ranging from polar to tropical regions) means that the environmental stressors experienced by developing fish eggs are extremely broad. River-spawning salmonids in temperate regions prefer warmer-than-average sites that minimize the likelihood of eggs freezing (Geist et al. 2002), whereas species in more tropical latitudes prefer cooler sites to prevent eggs from overheating (Middaugh et al. 1981). Many stream-dwelling fishes select spawning sites with sufficient water flow to aerate eggs and

prevent suffocation (Bilkovic et al. 2002), but occasionally, as in Eurasian perch (*Perca fluviatilis*), spawning sites with higher turbidity may actually be preferred because eggs deposited in such sites will experience reduced UV radiation (Probst et al. 2009). Substrate attributes, such as vegetation and grain size, are important because they provide sites for anchoring eggs, and thereby prevent scouring or washing away of eggs by currents or wave action (Shirotori et al. 2006).

Protection against predation is another important component of how fish maximize offspring survival via oviposition-site choice. Substrate composition, and especially the size of interstitial spaces between particles, is important in protecting fish eggs against predation (Middaugh et al. 1981), cannibalism (Spence et al. 2007), or disturbance by other fish spawning in the same location (Geist et al. 2002). Some galaxiids remove the threat of aquatic predators from their eggs altogether by depositing eggs terrestrially amongst flooded twigs and leaves (Charteris et al. 2003).

Many fishes exhibit parental care of eggs. In such species, the efficiency of parental care can be increased by selecting a favorable oviposition site. For example, nest-guarding male convict cichlids (*Cichlasoma nigrofasciatum*) that spawn in burrows could more effectively defend eggs against predation when the burrows had one, rather than multiple, entrances (Lavery 1991). Longfinned gobies (*Valenciennea longipinnis*) that spawned in burrows containing underground water flow reduced parental burden because the flowing water provided dissolved oxygen and thereby reduced the amount of egg-fanning required by the attending male (Takegaki 2001). Similarly, male threespine sticklebacks (*Gasterosteus aculeatus*) nesting in concealed locations more efficiently fanned their eggs because they

spent less time involved in territorial encounters with other males than did males whose nests were in more exposed locations (Sargent & Gebler 1980).

Selection of oviposition sites in amphibians is strongly driven by pressure to reduce predation on eggs. Many species avoid ovipositing in water bodies containing predators (e.g., Resetarits & Wilbur 1989,Vredenburg 2004). Similarly, in species with cannibalistic larvae, adults avoid ovipositing in pools already containing conspecific larvae (Matsushima & Kawata 2005). However, some tropical frogs prefer to oviposit at sites containing conspecific eggs or larvae, and may use the presence of conspecifics as indicators of predator absence (Rudolf & Rödel 2005).

Unlike fish, amphibians are not necessarily aquatic, and therefore are not all obligated to spawn and oviposit in an aquatic environment. But like fish, amphibian eggs lack a protective shell, rendering the eggs extraordinarily sensitive to environmental conditions, particularly moisture levels. Species that oviposit terrestrially, including many salamanders, must select oviposition sites with sufficient water content so that eggs do not desiccate (Figiel & Semlitsch 1995), while stream-dwelling species are challenged to select oviposition sites with greater stability to reduce nest displacement during high-flow events (Guy et al. 2004). Conversely, in species that oviposit in temporary pools, hydroperiod length is important because females must assess whether a potential oviposition-site will contain water long enough for eggs to develop and larvae to escape before the pool dries (Rudolf & Rödel 2005).

Thermal conditions are also important in maximizing embryo survival in amphibians. Freezing can be problematic for species that breed early or late in the activity season; to compensate, many pool-nesting species select deeper, rather than shallower, oviposition sites

(Petranka & Petranka 1981). Interestingly, a few species exhibit plasticity in nesting behavior depending on temperature: individuals may oviposit in isolation in warm weather but communally in cool weather (Caldwell 1986), which appears to confer a thermal advantage to developing embryos and results in higher embryo survival (reviewed in Doody et al. 2009). Amphibians also maximize embryo survival through oviposition-site choice by avoiding sites containing high salinity (Haramura 2008) and high transparency of water to damaging UV radiation (Palen et al. 2005).

In many reptiles, oviposition sites are selected to minimize predation on the developing eggs (e.g., Rand & Dugan 1983). In some cases, however, females must choose between minimizing predation on their nests or on themselves, known as the fecundity-survival hypothesis. For example, females in a population of tropical pythons (*Liasis fuscus*) chose between two types of nest locations: cool sites, which carry a lower risk of egg predation but require costly brooding by the female; and warm sites, which do not require maternal brooding but have a higher risk of egg predation (Madsen & Shine 1999). Females that nested in cool sites were emaciated following the nesting season, and many later died of starvation. The high costs to females associated with selecting cool nest sites suggest that they favored survival of their offspring over their own survival (Madsen & Shine 1999).

While reptile eggs are more protected from environmental conditions than are eggs of species lacking calcified egg shells, such as amphibians and fish, selecting an oviposition site that will experience conditions suitable for embryonic development is nonetheless important. Incubation temperature has a profound effect on embryo survival (e.g., Schwarzkopf & Brooks 1987), which is thought to be more important in nest-site choice than sex ratio adjustment in species with temperature-dependent sex determination (TSD; see Hypothesis 3

below; Ewert et al. 2005). In species from temperate regions where neonates overwinter in the nest, offspring survival is further influenced by the winter conditions they experience within the nest (Weisrock & Janzen 1999). Crocodilians have a particularly restricted range of suitable incubation temperatures, and offspring survival is low in areas that lack sufficient open, sunny sites for nest mound construction (Leslie & Spotila 2001). Soil moisture also strongly affects embryo survival in reptiles, and respiratory gas concentrations may be important as well, but few data are available on their impact on reptilian embryos in nests (Packard & Packard 1988). Eggs can desiccate in soil that is too dry (Socci et al. 2005); moreover, reptiles with flexible-shelled eggs must absorb some moisture from the environment to successfully complete development (Packard & Packard 1988). However, eggs in nests that are too moist are susceptible to fungal infection (Socci et al. 2005) or drowning. The risks to nests of many beach-nesting reptiles, such as sea turtles, commonly differ depending on a nest's location: nests closer to water are at highest risk of inundation or egg loss due to erosion, while eggs farther from water are at highest risk of desiccation (Kamel & Mrosovsky 2004).

Predation is the highest source of nest mortality in passerine birds (Martin 1992), and the selection of nest sites that reduce the risk of predation is thought to be the predominant driver of nest-site choice in birds in general (Martin 1993). Many birds minimize nest predation by selecting nest sites that are concealed by vegetative or other cover and are therefore less detectable to predators than are random sites (e.g., Liebezeit & George 2002). However, many studies found no relationship between nest concealment and likelihood of predation (reviewed in Martin 1993). In these cases, birds may reduce predation through parental presence at the nest, either during incubation or the period of parental care in species

with altricial nestlings (the parental-compensation hypothesis; Remeš 2005). Nest predation can be further reduced in colonially-nesting species where each individual benefits from the increased vigilance of numerous adults. Some species, particularly shorebirds, take advantage of the vigilance and predator-mobbing behavior of colonially-nesting species and place their nests within a colony of another species, thereby increasing their own nesting success (Pius & Leberg 1998).

However, parental presence at the nest can be costly in birds, as incubating adults are vulnerable to predation and unfavorable environmental conditions. Evidence supporting the fecundity-survival hypothesis in birds is mixed (Miller et al. 2007), but a review of North American bird species suggested that, in general, adults of the reviewed species acted to reduce predation risk to their offspring over reducing their own predation risk (Ghalambor & Martin 2001). In particular, a tradeoff exists between concealing the nest from the view of predators, and maintaining the incubating parent's view of the surroundings (Götmark et al. 1995), which may explain why increased nest concealment does not always result in decreased nest predation in birds (e.g., Holway 1991).

Because most bird nests are constructed in the open (i.e., they are not buffered by water or soil), the developing eggs and nestlings are particularly vulnerable to environmental stressors such as extreme heat or cold. Birds whose nests are exposed to particularly high temperatures, such as grassland- or beach-nesting species, must select nest sites that minimize heat stress to eggs and nestlings. Nesting amongst patches of vegetation (Davis 2005) or orienting the nest opening away from the sun during the hottest part of the day can reduce direct solar radiation on eggs and nestlings (Facemire et al. 1990). On the other hand, species from cold climates may select sites that increase solar radiation (Marzluff 1988) or

have a decreased risk of being covered by snowdrift (Stonehouse 1970). Megapodes construct nest mounds from which the young emerge fully independent of adults, and these birds select mound sites with thick vegetative cover to prevent desiccation of the incubating eggs (Jones 1988).

Studies on a few passerine birds have documented a generalist strategy, wherein no specific nest-site variables were predictive of nest success or failure (e.g., Filliater et al. 1994). Instead, a rich guild of nest predators may eliminate predictably safe nest sites, and thereby select for generality over specificity in nest-site choice. Alternatively, such species may not select nest sites to maximize embryo survival, but instead for one of the reasons discussed below.

Hypothesis 2: Maximizing maternal survival

Maximizing maternal survival is important in any oviparous animal, but it is of particular importance in iteroparous species where the lifetime reproductive success of a female depends on her survival across periodic breeding events. In species where some form of parental care occurs (e.g., nest attendance in salamanders; egg incubation in birds), the period of maternal vulnerability while at the oviposition site extends beyond oviposition itself, and lasts throughout the period of parental care. This observation has given rise to the fecundity-survival hypothesis, where females must choose between increasing their fecundity despite the concurrent increase in mortality risk to themselves and reducing their own mortality risk at a cost to their offspring.

The preference-performance hypothesis for insects states that females should prefer to oviposit on hosts with the highest nutritional quality for offspring, which would promote

short development time, higher adult biomass, and increased maternal fecundity (see Hypothesis 4 below; Pöykkö 2006). However, oviposition-site choice in many phytophagous insects fails to support this hypothesis: in the pierid butterfly *Anthocharis cardamines*, females oviposited on a host plant that provided poor nutrition for the larvae and did not oviposit on the plant on which larvae had the highest performance (Courtney 1981); similarly, a grass miner (*Chromatomyia nigra*) optimized the number of eggs laid per host plant rather than optimizing the quality of the host plant selected for the larvae (Scheirs et al. 2000). Rather than optimizing habitat quality for the offspring, such species are instead optimizing maternal fitness and/or survival. Female *A. cardamines* fed extensively on the nectar of the plant they selected for oviposition (Courtney 1981), and the grass miners optimized their own fecundity over the performance of their offspring (Scheirs et al. 2000). Selection of oviposition sites that are sub-optimal for offspring may also result from time limitation, rather than egg limitation in many insects: that is, time-limited females should be less choosy about where they oviposit than egg-limited females, because the former simply want to lay all of their eggs before they die (Rosenheim et al. 2008). Therefore, suitable host plants may be chosen for oviposition simply to maximize maternal fecundity, even though those host plants are not ideal for offspring performance.

In fish and amphibians, where external fertilization requires that both parents be present at the time of oviposition, minimizing predation on vulnerable mating pairs is an important consideration in choice of oviposition site. In many species of wrasse (*Thalassoma* spp.), females require information about the safety of a potential spawning site before they will mate with the resident male (Warner & Dill 2000). Similarly, the habitat patches used for spawning by river herring (*Alosa* spp.; O'Connell & Angermeier 1997) and

the rapid upward dashes characteristic of many spawning tropical coastal fish (Johannes 1978) both minimize predation on spawning adults. In amphibians, the unique posture adopted by a mating pair of anurans (i.e., amplexus) has metabolic and locomotor costs (Bowcock et al. 2009), and is also likely to increase the pair's vulnerability to predation, which may influence where a pair chooses to oviposit. Female salamanders that brood egg masses incur metabolic costs and probably a higher direct risk of mortality as well (e.g., Forester 1981).

Because nesting reptiles generally provide little, if any, parental care, and mating is spatially and temporally separated from nesting due to internal fertilization, the mortality risk to nesting females is usually restricted to traveling to a nesting area and constructing the nest. For example, *Sceloporus* lizards selected warm nest sites in open areas that accelerated embryonic growth and development, but females nested at night to minimize risk of overheating at the nest site (Angilletta et al. 2009). Other species select nest sites that protect the nesting female from predation (e.g., Burger 1993). Nests of the turtle *Emydura macquarii* experienced lower predation rates when located farther from, rather than nearer to, the shoreline. However, when direct predation risk to females increased, the turtles favored their own survival by nesting closer to the shoreline at the expense of increased predation risk to the nest, supporting the fecundity-survival hypothesis (Spencer 2002). Females may also minimize energy expended on selecting and constructing nest sites by nesting communally (reviewed by Doody et al. 2009) or copying the nest-site choice of conspecifics (Refsnider et al. 2010).

The prolonged stage of parental care in birds, particularly during incubation, means that adults are vulnerable to the same risks as their developing offspring, namely predation

and environmental stressors. According to the microclimate-selection hypothesis, nest sites are chosen to minimize physiological stress on the incubating adult (With & Webb 1993). Studies of nest-site choice of individuals differing in body condition provide support for this hypothesis: in Kentish plovers (*Charadrius alexandrinas*), females in poor body condition chose nest sites that were thermally less stressful for the female but had a higher risk of nest predation, whereas females in better body condition chose thermally more stressful nest sites with a lower risk of nest predation (Amat & Masero 2004). In several grassland passerines, whose nests are exposed to high temperatures and prolonged periods of direct sunlight, nest sites were chosen such that the degree of radiative cover provided shade and convective cooling to incubating adults, which is particularly important in dark-colored species (With & Webb 1993). A review of South American passerine species supported the fecundity-survival hypothesis, whereby parents acted to reduce predation risk to themselves over that of their offspring (in contrast to the behavior of North American passerines; see Hypothesis 1); moreover, this response increased as clutch size decreased (Ghalambor & Martin 2001).

While many birds select nest sites to minimize detection by predators, a cost of selecting a concealed nest site is that heavy cover obstructs the incubating parent's view of the surroundings (Götmark et al. 1995). In Canada geese (*Branta canadensis*), female survival was more dependent than nest survival on the incubating female's early detection of predators, and therefore a negative correlation existed between nest concealment and adult survival (Miller et al. 2007).

Hypothesis 3: Modifying offspring phenotype

In reptiles and, to a lesser extent, fish and amphibians, the micro-habitat in which a female oviposits can dramatically affect the phenotype of her offspring. Incubation regime influences numerous fundamental characteristics, such as duration of embryonic development, offspring size, post-hatching growth rate, locomotor performance, and behavior. In addition, in species with TSD, thermal characteristics of the nest site determine offspring sex ratio. Therefore, choice of oviposition site directly affects not only the survival of a female's developing embryos, but also the quality of her offspring.

Because eggs of fish and amphibians are generally unattended after oviposition, the incubation conditions they experience during development are solely those of the oviposition site. Warmer conditions accelerate embryonic development in fish (Bermudes & Ritar 1999), amphibians (Pearl et al. 2007), and reptiles (Shine et al. 1997). This relationship is especially important in species where early hatching is favored, such as frogs whose larvae must escape from drying ephemeral pools or temperate-zone reptiles with limited time in which to acquire sufficient reserves before entering hibernation.

Moisture level and thermal conditions (including both means and variances) also affect numerous other offspring characteristics in reptiles. Moisture level and incubation temperature affected offspring size in a snake (e.g., Brown & Shine 2004), and the effects of incubation temperature on growth rate in snapping turtles (*Chelydra serpentina*) persisted for at least 7 months after hatching (Brooks et al. 1991). Incubation conditions affect several measures of offspring performance including metabolic efficiency in lizards (Van Damme et al. 1992), swimming speed in turtles (Miller 1993), and sprint speed in lizards (Shine et al. 1997). Offspring behaviors such as basking (Shine & Harlow 1996), retreating from

predators (Burger 1989), and sociosexual behaviors (Flores et al. 1994) also vary significantly along a gradient of incubation conditions.

Many reptiles, and a few species of fishes, have TSD, whereby offspring sex is irreversibly determined by the incubation temperature within the nest cavity (Bull & Vogt 1979). Therefore, TSD potentially affords reptiles control over the offspring sex ratio via nest-site choice (Janzen & Morjan 2001). By choosing a nest site to produce a specific sex, a female theoretically could increase her fitness by producing the rarer, and thereby more valuable, sex (Girondot et al. 1998); the sex that will benefit more from a higher-quality nest-site (Reinhold 1998); or the sex whose fitness is maximized by the incubation regime that produces that sex (Conover 1984; Warner & Shine 2008). Therefore, while nest-site choice in TSD reptiles may be driven predominantly by selecting a site that maximizes nest success (as offspring phenotype is meaningless if the eggs fail to hatch; Escalona et al. 2009), optimizing offspring phenotype is likely the next most important reason for selecting a particular site (Ewert et al. 2005).

Hypothesis 4: Proximity to suitable habitat for offspring

Larval and juvenile animals are often especially vulnerable to predation, desiccation, and/or starvation. It is therefore critical that, immediately following hatching, juveniles locate suitable habitat in which to hide from predators and obtain sufficient nutrients for growth and development. In insects, females that oviposit on ideal host plants decrease the length of time their offspring spend in the vulnerable larval stage. Similarly, salamanders that oviposit in deeper temporary ponds increase the chances that their offspring will complete larval development and escape from their ponds before they dry up. In species that

oviposit in different habitat from that required by juveniles immediately after nest emergence, offspring survival may be enhanced if females oviposit in close proximity to the habitat required by juveniles. Examples include hatchling turtles moving from their terrestrial nest site to appropriate aquatic habitat, or fledgling birds that must quickly find suitable foraging habitat or risk starvation.

The preference-performance hypothesis for phytophagous insects states that females should prefer to oviposit on the host plants that confer the highest nutritional benefits to their larvae (Pöykkö 2006). Studies on many insect species support this hypothesis, which indicates that such species likely select oviposition sites to provide suitable habitat for their larvae. For example, larvae whose eggs were laid on hosts preferred by females grew larger (Rausher 1983), had shorter development time (Vacek et al. 1985), and had higher digestive efficiency (Sadeghi & Gilbert 1999) than larvae that hatched on hosts not preferred by ovipositing females. Nutritional quality is not the only benefit that larval insects may receive from their host plant, however. Female insects can also affect larval performance by ovipositing on host plants that allow larvae to sequester defensive chemicals (Thompson & Pellmyr 1991), support ant species that protect larvae from predators and parasitoids (Pierce & Elgar 1985), allow larvae to move easily among several host species and thus achieve a mixed diet (Ballabeni et al. 2001), give larvae sufficient time to complete development before the host plant dehisces (Wiklund & Friberg 2009), and minimize intraspecific competition on the host plant (Rausher 1983). Females of carnivorous species, such as pitcher plant mosquitoes (*Wyeomyia smithii*), preferred to oviposit in larger pitchers, which contained more prey for their larvae and therefore facilitated decreased larval development time and increased size of larvae (Heard 1994).

Insect larvae that develop in temporary pools are at risk of predation, interspecific competition, and desiccation from pool drying. Mosquitoes (*Culiseta longiareolata*) avoided ovipositing in pools that contained predators of their larvae (Spencer et al. 2002), thus ensuring safer habitat for their offspring. Similarly, where larval mosquitoes (*Anopheles punctipennis*) compete with species such as anuran tadpoles for food resources, female mosquitoes avoided ovipositing in pools containing the competitor species (Petranka & Fakhoury 1991). A pool that lacks predators or interspecific competitors is often temporary; therefore, larvae must complete development and escape before the pool dries (Blaustein & Margalit 1996), which favors maternal ability to assess a pool's hydroperiod.

The spawning site can affect the transport of eggs and/or larvae in many fishes. Temperate, stream-dwelling species may spawn in upstream reaches, which contain both suitable conditions for embryonic development and fast currents that transport larvae to downstream environments rich in food resources (Bilkovic et al. 2002, Charteris et al. 2003). Many coral reef fishes spawn at times and locations that favor transport of pelagic larvae offshore, where predation is drastically reduced (Johannes 1978). Conversely, tropical pelagic species spawn at sites such that tides or currents transport eggs reefward, where larvae can develop in protected, food-rich sites (Leis 1991). Increasingly, however, we recognize that the larvae of many coral reef fishes are behaviorally sophisticated rather than passive plankters (Leis 1991), which suggests that these larvae play an active role in arriving at suitable habitat rather than relying on currents in the vicinity of spawning grounds to transport them passively.

Due to the larval stage of taxa with indirect development, amphibian tadpoles face many of the same pressures as larval fish. Selecting pools that minimize predation (e.g.,

Resetarits & Wilbur 1989), cannibalism (Halloy & Fiano 2000), and intraspecific competition (e.g., Matsushima & Kawata 2005) among tadpoles is of primary importance in oviposition-site choice in many anurans. Male Madagascan poison frogs (*Mantella laevigata*) select oviposition sites and then call to attract females to those sites; however, males actively discriminated against potential oviposition sites already containing competing frog species (Heying 2004). Similarly, gray treefrogs (*Hyla versicolor*) avoided ovipositing in pools containing high densities of parasitic snails (Kiesecker & Skelly 2000).

Like larvae of insects that oviposit in temporary pools, amphibian larvae developing in an ephemeral water body must metamorphose and escape before a pool dries (Blaustein & Margalit 1996). Females may therefore prefer to oviposit in pools with longer hydroperiods to increase the likelihood that tadpoles can develop and metamorphose before drying occurs (Resetarits & Wilbur 1989). Stream-dwelling species, such as *Desmognathus* salamanders, nested in headwater habitats that passively transported larvae into optimal juvenile habitat in moderate-sized streams, while minimizing transport to suboptimal habitats in large rivers (Snodgrass et al. 2007), similar to the larval transport mechanisms observed in many fishes.

Choice of nest sites in areas that reduce predation on newly-hatched offspring is known in some reptiles. Nests laid by green iguanas (*Iguana iguana*) and slider turtles (*Trachemys ornata*) on an island were subject to much less predation on the resulting hatchlings compared to nests laid on nearby mainland habitat (Drummond 1983), and hatchling snapping turtles (*C. serpentina*) were more likely to survive the journey from nest to wetland if the nest site was characterized by sparse vegetation, little slope, and was in close proximity to water (Kolbe & Janzen 2001). Upon emergence from the nest, sea turtle hatchlings must orient correctly in order to reach the ocean. Female leatherback

(*Dermochelys coriacea*) sea turtles selected nest sites at an intermediate distance from the high tide line, where hatchling disorientation was less likely than at sites farther from the water (Kamel & Mrosovsky 2004). On a larger spatial scale, female sea turtles may select nesting beaches that are in close proximity to ocean currents that will transport hatchlings to suitable developmental habitats (Lohmann et al. 2008).

Upon fledging, young birds lose the protection provided by their nest against predation and environmental stressors. Parents of many species, therefore, often lead newly-fledged offspring away from the vicinity of the nest and into different habitat that provides food (Vega Rivera et al. 1998) and concealment from predators (Anders et al. 1998). Habitat that provides food and protection is important for both altricial species where fledglings continue to be fed by the parents, as well as precocial species in which young are completely independent upon hatching (Göth and Vogel 2002). The necessity to quickly lead vulnerable young from the nest site to suitable fledgling habitat favors parental selection of nest sites that are close to fledgling habitat (H.M. Streby & D.E. Andersen, unpublished data). Indeed, in cases with no correlation between nest-site variables and nest survival (e.g., Misenhelter & Rotenberry 2000), nest sites may be selected on the basis of proximity to suitable fledgling habitat rather than for the nest microhabitat. Because an individual's lifetime fitness is based on reproductive success (which in birds includes both nest success and survival of fledglings until independence), rather than nest success per se, it would not be surprising if proximity to suitable fledgling habitat was as important in driving nest-site choice in birds as maximizing nest success. Research on the fledgling stage of birds is necessary to test this idea. Other taxa that move their offspring, such as some dendrobatid frogs that transport tadpoles

(Aichinger 1991), may also choose their initial oviposition site on the basis of proximity to suitable habitat to which adults can transport juveniles.

Hypothesis 5: Maintaining natal philopatry

In some taxa, oviposition-site choice may be an artifact of natal philopatry, with females returning to nest at the same location where they themselves hatched. If oviposition-site choice is heritable, females that oviposit at sites that produce high-quality offspring would pass on this oviposition-site choice to their daughters, thereby maintaining a lineage that produces high-quality offspring resulting from oviposition-site choice. While maintaining natal philopatry is probably less important in driving oviposition-site choice than the hypotheses described above, it nevertheless affects where females of some species choose to oviposit.

Selection favors philopatry when dispersal from natal habitat entails high costs. For example, in damselflies (*Enallagma* spp.) that are unable to determine if a potential oviposition site contains predators, females are strongly philopatric and thus maintain within their lineage daughters that continue to oviposit in the habitat in which they themselves hatched (McPeek 1989).

In contrast, dispersing species may display natal philopatry to ensure that daughters return to, and oviposit at, successful nest sites. Such philopatry may be passive, as in the case of many tropical fishes that spawn near gyre currents that periodically return larvae to the vicinity of origin (Johannes 1978); or daughters may actively return to the site of their own hatching, as noted in northern spectacled salamanders (*Salamandrina perspicillata*; Romano et al. 2008) and sea turtles (e.g., Meylan et al. 1990). Under this active-return

model, a dispersing female initially selects a nest site based on characteristics that influence offspring survivorship, and then returns to that site on subsequent nesting forays as long as the site retains the features for which it was selected; the natal nest area may be the first site chosen under this model (Lindeman 1992). Particularly in unpredictable environments, nesting success in the previous generation may be the best predictor of future success, so inheritance of oviposition-site preference would provide a mechanism by which a female could identify a suitable oviposition site and then transmit that information to her female descendents (Freedberg & Wade 2001). Moreover, imperfect natal homing could result in occasional colonization of a new oviposition habitat, thereby fostering gene flow and providing an alternative oviposition site if previously-used sites become unsuitable (Bowen et al. 1989).

Support for the benefits of natal philopatry in birds is somewhat mixed, and depends mainly on the quality of territory in which an individual hatches (Stacey & Ligon 1991). On the one hand, a female breeding for the first time benefits from searching a familiar, high-quality area for a suitable nest site (Emlen 1994), and the fact that a nest site has already been successful indicates that nesting as close as possible to her natal site is a safe strategy (Ruusila et al. 2001). On the other hand, in areas lacking a necessary resource, females benefit by remaining in their natal territory, helping relatives to breed, and waiting to inherit the parental territory (Emlen 1994). Natal philopatry is also beneficial in species such as waterfowl where intraspecific brood parasitism and/or post-fledging brood amalgamation occur, as the costs of these behaviors decrease when performed among relatives (Weatherhead 1998).

The degree to which natal philopatry occurs may vary between individuals and between populations of the same species. For example, about 70% of California tiger salamanders returned to breed at the pond in which they had metamorphosed, while about 30% dispersed and bred at a different pond (Trenham et al. 2001). Is such variation a population strategy to maintain gene flow among habitat patches, a behavioral syndrome wherein some individuals are always dispersers while others always show natal philopatry, within-individual behavioral plasticity based on environmental conditions, or are dispersing individuals simply showing imperfect natal homing? Determining the mechanism(s) driving natal philopatry will give us insight into both its adaptive significance in general, and its role in oviposition-site choice specifically.

Hypothesis 6: Indirect oviposition-site choice via mate-choice

In some cases, females may be able to affect the phenotype of their offspring indirectly through mate-choice. For example, in fish where males construct nests and/or tend eggs, the quality of the spawning site may be correlated with the quality of the male tending that site. Therefore, if offspring from eggs deposited in high-quality oviposition sites are more likely to be sired by males with high-quality phenotypes, the genetic quality of a female's offspring are within the female's control via her selection of an oviposition site. Indeed, in a review of resource-quality and male reproductive success, males with higher resource-holding potential controlled better territories in insects, amphibians, and birds (Kelly 2008).

In several fishes displaying paternal care of eggs, characteristics such as size are reliable indicators of a male's quality. For example, larger males are less likely to abandon

the nest, experience less egg loss to predation or cannibalism, and control larger nests that accommodate more eggs (e.g., Nelson 1995). Females that mate with these higher-quality males therefore achieve not only better genes for their offspring via mate-choice (Jones 1981), but also increase their offspring's likelihood of survival. Similarly, in bullfrogs (*Rana catesbeiana*), larger males controlled territories with lower predation risk than did smaller males, so females increased survival of their tadpoles by mating with a larger male and ovipositing in his territory (Howard 1978).

Males of many bird species set up nesting territories to which they attempt to attract females. Most studies attempting to disentangle mate-choice from nest-site choice in birds have concluded that females chose a male based on the quality of his territory rather than the reverse (e.g., Alatalo et al. 1986). However, as males controlling better territories tend to be higher-quality males, females that select higher-quality mates on the basis of territory quality are nevertheless passing on better genes to their offspring than females mating with lower-quality males.

Conclusions

The ultimate reasons for an individual's choice of oviposition site vary widely both among and within oviparous taxa. Such variation, particularly that occurring within one of the taxonomic groups discussed above, emphasizes the importance of hypothesis-testing in studies of oviposition-site choice rather than generalizing based on results from other taxa. Research on several taxonomic groups has traditionally focused on a specific hypothesis for oviposition-site choice within that group, to the exclusion of other possible explanations. For example, a plethora of studies has examined oviposition-site choice in insects in the context

of testing the preference-performance hypothesis, while in fact the oviposition-site choices of many insects do not support this hypothesis. Similarly, numerous studies of nest-site choice in birds fail to find relationships between nest-site characteristics and nest success. While such studies effectively eliminate the tested hypothesis as an explanation for oviposition-site choice in the species of interest, the next logical step is to test other hypotheses, one of which will likely explain the observed oviposition-site choice. For example, many insects select oviposition sites that maximize maternal, rather than offspring, survival and/or performance, and recent research on the post-fledging period in birds suggests that suitability of habitat for fledglings is as important as nest success in determining a parent's reproductive success. The hypotheses discussed herein are not mutually exclusive, and it is likely that females in some taxa select oviposition sites hierarchically, based first on one hypothesis (such as maximizing female survival) and secondarily on another hypothesis (such as maximizing embryo survival) within the context of the first. The relative importance of each hypothesis is likely to differ among taxa, however, so even hierarchies of oviposition decisions should not necessarily be extrapolated across taxa.

Research on oviposition-site choice increasingly focuses on its relationship with anthropogenic changes to the environment, such as climate change, habitat loss, and the widespread introduction of invasive species. Habitat destruction and fragmentation are likely to eliminate habitats required by certain species for oviposition and thereby reduce successful recruitment. Invasive species may compete with native species for oviposition sites, replace native host species of some insects, or alter the microclimate of oviposition sites. Finally, climate change not only will exacerbate habitat loss and the establishment of invasive species, but also has the potential to directly alter the phenotypic composition of populations,

such as skewing sex ratios in species with TSD. It is therefore important to understand both the ultimate reasons for an individual's selection of an oviposition site, as well as the proximate cues used to select a site from a range of potential sites.

Perhaps most importantly in the face of global environmental change, it is also critical to understand the adaptive potential of oviposition-site choice – that is, how likely is it that a given species can shift its oviposition-site choice to compensate for changes in the environment? Important insight into this question can be gained by examining oviposition-site choice across the geographic range of widespread species. By examining patterns in oviposition-site choice across space, we gain power to predict the capacity for temporal response to environmental change (Doody 2009). Species could potentially shift oviposition-site choices to adapt to environmental change via a shift in allele frequencies (which would require genetic variation for oviposition-site choice) and/or via behavioral plasticity (which would require possessing a range of potential responses to environmental cues). Determining the mechanism(s) by which geographic variation in oviposition-site choice is maintained would aid in predicting adaptive potential in response to environmental change, and thus in determining vulnerability to threats such as climate change, habitat loss, and invasive species.

Acknowledgments

The literature examining oviposition-site choice in animals is vast, and due to space constraints we are able to cite only a small fraction of the relevant papers on this topic. Many thanks to the following people for enlightening discussion of the topic and comments on earlier versions of the manuscript: A. Bronikowski, C. Chandler, G. Cordero, P. Dixon,

C. Kelly, T. Mitchell, A. Sethuraman, H. Streby, E. Takle, R. Telemeco, and D. Warner. Funding was provided by the Iowa Academy of Science and the Department of Ecology, Evolution & Organismal Biology at Iowa State University (to J.M.R.) and NSF DEB-0640932 (to F.J.J.).

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Definition of key terms

1. **Active-return model for natal philopatry** – A female selects a nest site based on characteristics that influence offspring survival, and then returns there during subsequent reproductive bouts as long as the site remains suitable. Although the

female could fix on a new nest site if a previously-used site becomes unsuitable, the natal nest site is likely to be the first site chosen under this model.

2. **Fecundity-survival hypothesis** – Parents face a tradeoff between responding to the risk of mortality directed towards their offspring versus towards themselves.
3. **Intraspecific brood parasitism** – A female lays eggs in the nest of another female of the same species without subsequently caring for the eggs or young. In birds, this reproductive strategy is particularly common in waterfowl.
4. **Microclimate selection hypothesis** – Nest placement in birds functions to ameliorate adverse microclimatic effects on eggs, nestlings, and/or incubating adults.
5. **Natal philopatry** – Individuals return to the location of their birth to breed and/or deposit eggs.
6. **Parental-compensation hypothesis** – Nest concealment alone may be insufficient to mitigate against predation pressure, and parents compensate for remaining risk by their presence at the nest via plumage camouflage, anti-predator behavior, etc.
7. **Post-fledging brood amalgamation** – Upon fledging from the nest, multiple broods converge to form temporary or permanent associations, and parents defend a common brood-rearing area. This behavior is common in precocial birds including waterfowl and shorebirds.
8. **Preference-performance hypothesis** – Female insects prefer to oviposit on host species that have the highest nutritional quality for their larvae to feed upon after hatching.
9. **Temperature-dependent sex determination (TSD)** – The sex of individuals is irreversibly determined by the temperature experienced by the developing embryo

during a thermosensitive period. This mechanism occurs in reptiles including tuatara, crocodilians, most turtles, and some lizards, as well as some fishes.

10. **Time- vs. egg-limitation** – A female insect's realized lifetime reproduction may be constrained by either the time available for locating suitable oviposition sites (time-limitation) or by her supply of mature eggs (egg-limitation).

Future directions

1. Many studies have examined oviposition-site choice to determine whether specific sites are chosen non-randomly. Such studies should also attempt to link oviposition decisions to fitness: that is, do chosen sites differ from random sites in parameters such as maternal survival, embryo survival, or offspring phenotype? Extending studies on oviposition-site choice to examine effects on fitness would give us insight into the adaptive significance of particular oviposition decisions.
2. Studies integrating both laboratory experiments and field observations, while not feasible in all circumstances, will go a long way towards illuminating the importance of specific variables in oviposition decisions and the subsequent effect on individual fitness. Experimentally manipulating specific variables will help identify the features of greatest importance in a female's selection of an oviposition site, which may be difficult to elucidate solely via observational study. However, careful observation of oviposition-site choice and its impact on fitness is crucial to pinpoint which variables are likely to be important in a female's selection of an oviposition site, and therefore which are worthy of more detailed experimental study.

3. Variation in oviposition-site choice in geographically widespread species can inform us about the adaptive significance and mechanistic basis of this trait. Such species are likely to experience different environmental conditions across their range, and may exhibit variation in oviposition-site choice behavior to match local conditions. Whether geographic differences in oviposition-site choice are primarily genetically-based due to local adaptation, or more behaviorally plastic across the range, has important implications for a species' capacity to compensate for rapid environmental changes such as climate change, shifting community structure, and the introduction of invasive species.
4. Future research should focus on testing Hypothesis 4 (proximity to suitable habitat for offspring). Many studies that find no effect of oviposition-site characteristics on survival of the eggs or nest may find an effect when those same characteristics are examined in relation to juvenile survival. Thus, proximity to suitable habitat for offspring is likely to be an underappreciated driver of oviposition-site choice. Studies testing this hypothesis are particularly lacking in birds, but additional research on insects, amphibians (such as dendrobatid frogs that transport tadpoles), and reptiles (especially sea turtles and model species such as *Anolis* lizards) would also be very illuminating.
5. Technical advances in molecular biology and radioisotope analysis have drastically improved our ability to study individual movements and population relatedness over wide geographic scales. In many systems, we are only just beginning to understand the extent to which individuals show natal philopatry and the mechanisms used in navigation and homing. Studies on reproductive ecology in any taxon should also

include research on whether natal philopatry occurs in the study species. In systems where natal philopatry is found, it would be very useful to examine the costs and benefits of philopatry and to determine whether philopatry is a behavioral syndrome, an adoptable strategy, or individuals making homing mistakes. Answering these questions could give particular insight in systems where not all individuals are philopatric and a comparative approach could be taken. This situation would provide valuable insight into the evolution of natal philopatry and its role in driving oviposition-site choice.

6. In taxa where mate-choice and oviposition-site choice are inter-related, particularly in fish, amphibians, and birds, researchers should attempt to disentangle whether females select oviposition sites directly and mates indirectly, or vice versa. In some taxa separating mate-choice from oviposition-site choice may be accomplished through a simple factorial design in which females are presented with combinations of high- and low-quality males and oviposition sites. This problem has particular implications for sexual selection research in groups such as birds where females could select a social mate based on the quality of his territory, but could also acquire the genes of a higher-quality mate through extra-pair fertilizations.
7. Very little research has been conducted on the costs of spawning in amphibians, aside from potential costs to females of multiple matings. Specifically, research on the costs of amplexus in anurans is necessary to assess the importance of maximizing maternal survival in the oviposition-site choice of amphibians.
8. Although research on reproduction in fish is particularly difficult to conduct compared to the other taxa discussed in this review, substantial advances have been

made in understanding the habitat requirements of juvenile and spawning adults of many game species. Comparable studies should be conducted on nongame species as well. Research is also needed on the habitat use and movement patterns of larval fishes, and particularly the role of oceanic currents in transporting juvenile fishes among different habitat types.

CHAPTER 3. BEHAVIOURAL PLASTICITY MAY COMPENSATE FOR CLIMATE CHANGE IN A LONG-LIVED REPTILE WITH TEMPERATURE-DEPENDENT SEX DETERMINATION

A paper accepted for publication in *Biological Conservation*

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Abstract

How are organisms responding to climate change? The rapidity with which climate is changing suggests that, in species with long generation times, adaptive evolution may be too slow to keep pace with climate change, and that alternative mechanisms, such as behavioural plasticity, may be necessary for population persistence. Species with temperature-dependent sex determination may be particularly threatened by climate change, because altered temperatures could skew sex ratios. We experimentally tested nest-site choice in the long-lived turtle *Chrysemys picta* to determine whether nesting behaviour can compensate for potential skews in sex ratios caused by rapid climate change. We collected females from five populations across the species' range and housed them in a semi-natural common garden. Under these identical conditions, populations differed in nesting phenology (likely due to nesting frequency), and in nest depth (possibly due to a latitudinal cline in female body size), but did not differ in choice of shade cover over the nest, nest incubation regime, or in resultant nest sex ratios. These results suggest that choice of nest sites with particular shade cover may be a behaviourally plastic mechanism by which turtles can compensate for change in climatic temperatures during embryonic development, provided that sufficient environmental variation in potential nest microhabitat is available.

Introduction

Numerous studies have documented effects of climate change on natural systems, including range shifts, changes in phenology, altered species interactions, and disrupted community structure (reviewed in Parmesan, 2006). A growing question is whether, and to what extent, organisms may be able to adjust to climate change, and by what mechanism(s) such adjustment may be possible. At the population level, adaptive genetic changes in response to climate change have been observed in a few cases (e.g., Bradshaw and Holzapfel, 2001; Umina et al., 2005). However, due to the rapid pace at which global climate change is occurring, mechanisms occurring at the individual level, such as behavioural plasticity, may be more feasible for adjusting to novel climatic conditions (e.g., Berteaux et al., 2004). Although behaviourally-plastic traits may not necessarily have a strong genetic basis, in some situations behavioural plasticity may vary adaptively in response to environmental variability, and therefore could occur simultaneously with genetic adaptation to climate change.

Global temperatures are predicted to increase as much as 6.4^{o}C by 2100 (Solomon et al., 2007). Long-lived species could be particularly vulnerable to a change of this magnitude because a lag may develop between environmental conditions and optimum trait values. Although such a lag could lead to a fast evolutionary response due to intense directional selection, it is also possible that the genetic variability available to selection might decrease rapidly under strong directional selection, resulting in extinction rather than evolution if environmental conditions change too quickly (Hoffman and Sgrò, 2011). Shorter-lived species with more generations per time-period are less likely to develop a lag between environmental conditions and optimum trait values, and therefore should be able to adapt to

climate change more quickly than long-lived species (Dunham and Overall, 1994; Pen et al., 2010).

The outcomes of climate change on biota are difficult to predict because they involve indirect effects of environmental changes on a multitude of organisms and occur via complex pathways. However, some thermally-sensitive traits exist that are directly impacted by climate and have demographic consequences for populations. One such trait is temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos. TSD occurs in some invertebrates and fishes, and it is common among long-lived reptile groups. In species with TSD, the sex-determining pathway is extremely sensitive to temperature: the transitional range of temperatures, within which the complement of offspring sex shifts from 100% male to 100% female (or vice versa), is generally less than 2^{o}C, and may be less than 1^{o}C (Ewert et al., 1994). This degree of thermal sensitivity means that a small change in environmental temperature could dramatically alter offspring sex ratio, and potentially result in populations consisting of predominantly one sex (Janzen, 1994a; Nelson et al., 2004).

Reptiles with TSD have survived past periods of dramatic global warming and cooling. The persistence of such taxa despite past periods of climatic change could be explained either by changes in the specific incubation temperatures producing each sex, or by changes in maternal nest-site choice, which affects the microclimate experienced by developing embryos (Bulmer and Bull, 1982). Although incubation temperatures and maternal nest-site choice are likely to exert an impact over evolutionary timescales, low heritability suggests that shifts in thermal sensitivity of sex determination are unlikely to compensate for skewed sex ratios resulting from rapid climate change (Janzen, 1994a; but

see McGaugh and Janzen, 2011). Moreover, seemingly minimal phenotypic variation in this sex-ratio reaction norm between populations suggests that the sex-ratio reaction norm has provided an insufficient target for selection to compensate for climatic differences (Morjan, 2003a). At the same time, micro-evolutionary responses of nest-site choice to a rapidly changing climate appear to be context-specific at best (McGaugh et al., 2010) and ineffective at worst (Morjan, 2003b). Therefore, if long-lived reptiles with TSD are to avoid skewed sex ratios produced by temperatures changing at unprecedented rates, behavioural phenotypic plasticity in nest-site choice (i.e., the ability of individual females to intra-generationally alter the location or other characteristics of nests in response to environmental conditions) may be the likely immediate mechanism (Morjan, 2003b). Importantly, such a result would contrast with the micro-evolutionary response to climate change observed in short-lived taxa (e.g., Bradshaw and Holzapfel, 2001; Umina et al., 2005; Balanyá et al., 2006; Pen et al., 2010).

We examined nest-site choice in a model long-lived turtle with TSD, *Chrysemys picta*. We used a common-garden design to determine whether local adaptation of key elements of nest-site choice is more intrinsically- (e.g., genetically canalized) or environmentally-based. In several reptiles with TSD, maternal choice of oviposition site exhibits local adaptation, with females selecting different nest sites to match incubation conditions to their latitude (Ewert et al., 2005; Doody et al., 2006a). However, whether patterns of geographic variation in nest-site choice are driven by inheritance of specific nesting behaviour, or by phenotypic plasticity, is unknown. The elements of nest-site choice we examine here are nesting date, shade cover over the nest site, and nest depth. Although the mechanisms controlling these elements differ (e.g., nest depth is influenced by clutch size and body size [Tiwari and Bjorndal, 2000], while nesting date is likely mediated by

environmental effects on the endocrine system [Wingfield, 1983]), each element has been shown to vary in response to environmental conditions in other reptiles with TSD (e.g., nesting date: Zani, 2008; shade cover: Doody et al., 2006a; nest depth: Telemeco et al., 2009). By examining patterns of variation in nest-site choice across space, our experimental design allows us to infer the processes of such variation through time, and thereby predict a species' capacity to respond to climate change via nest-site choice.

1. Materials and methods

The painted turtle is widely distributed in freshwater habitats across the U.S. and southern Canada. The western subspecies, *C. p. bellii*, was used in this study and primarily occurs west of the Mississippi River. Populations of this subspecies are genetically similar and are clearly differentiated from the eastern subspecies (Starkey et al., 2003). Female painted turtles emerge from wetlands between May and July to nest in a variety of open habitats ranging from beaches to lawns. Individual females nest from 1 to 3 times per season, varying among populations. Painted turtles exhibit TSD, with females produced at constant incubation temperatures above 29^{o}C and males produced at constant temperatures below 27^{o}C (Ewert et al., 1994). Age at maturity of female *C. picta* at the Illinois study site is 5 – 8 years (Bowden et al., 2004; Spencer and Janzen, 2010).

We captured adult *C. p. bellii* between 27 April and 27 May 2009 from 5 populations across the subspecies' U.S. range: Socorro Co., NM (33^{o}46'15"N, 106^{o}54'07"W); Carroll Co., IL (41^{o}57'23"N, 90^{o}07'49"W); Grant Co., NE (41^{o}59'47"N, 101^{o}44'05"W); Story Co., IA (42^{o}03'45"N, 93^{o}37'49"W); and Skamania Co., WA (45^{o}37'42"N, 122^{o}00'52"W; Supplementary figure 1). The order in which turtles were collected and released into the

common-garden environment was NM first, followed by the three central populations, and finally by turtles from WA. We obtained 13, 15, 6, 15, and 11 adult females from the NM, IL, NE, IA, and WA populations, respectively, using a variety of aquatic trapping methods. We measured all turtles (straight and curved carapace length and width; plastron length and width; length of rear limbs) and marked individuals by filing a unique combination of notches into the marginal scutes.

We housed turtles in a series of identical, outdoor, semi-natural ponds at Iowa State University's Aquatic Research Facility (Story Co., IA; Supplementary figure 2). Each population occupied a separate 15 x 40 m pond, each of which was surrounded by a 0.5 m-high drift fence to prevent escape of experimental turtles or entrance of local turtles. Ponds graded from 2 to 3 m deep from north to south, and were filled with water from an adjacent lake approximately 3 weeks before release of the experimental turtles to allow colonisation by local aquatic plants, invertebrates, and anurans, all of which are eaten by painted turtles. Turtles had ad libitum access to these food items, and were not provided with supplementary food (we monitored food availability and observed no evidence of food reduction throughout the study). Two basking logs were present in each pond. The drift fencing around each pond was set back ~8 m from the shoreline on all sides of the ponds to provide nesting habitat. In addition to the natural vegetation growing around the perimeter of the ponds (Supplementary figure 2), we provided additional artificial shading by placing 5, 3 x 3 m sections of shadecloth 1.5 m above the ground in randomly-assigned locations around each pond. These artificial shade objects were attached at the corners to PVC pipes driven into the ground. The amount of available shade around each pond spanned a gradient from 7% (on unvegetated banks) to 70% (under artificial shade objects) cover.

We monitored the ponds for nesting activity from 27 May to 3 July 2009. We patrolled the perimeters of all ponds hourly from 0600-1000 h and 1500-2100 h, the times of peak painted turtle nesting activity. Turtles that were observed nesting (Supplementary figure 3) were monitored from a distance of at least 10 m to prevent nest abandonment due to disturbance. Upon completion of nesting, we briefly detained females for identification, and then released them back into their ponds. We excavated all nests within 24 h of construction to determine clutch size and measure nest depth; we also recorded the date of construction and took a hemispherical photograph over the nest site to quantify shade cover using Gap Light Analysis software (as in Doody et al., 2006b), and inserted a data logger (iButtons, Embedded Data Systems, Lawrenceburg, KY) amongst the eggs to record nest temperature hourly throughout incubation. Nests then remained in situ for the duration of the incubation period.

In October 2009 (after hatching but before emergence from the nest), we excavated all nests to retrieve hatchlings and data loggers. We calculated hatching success as the number of live hatchlings retrieved, divided by the known clutch size. To assess sex, we sacrificed hatchlings with a pericardial overdose of 0.5 mL of 1:1 sodium pentobarbital:water and examined the gonads (Janzen, 1994b): individuals lacking oviducts and possessing short gonads were classified as males (1), those with complete oviducts and long gonads were classified as females (0), and individuals possessing both male and female gonads were classified as intersexes (0.5). After sexing, we preserved all specimens in 70% ethanol.

We acquired climate data in the form of mean daily temperature for each collection site from the National Climate Data Center (www.ncdc.noaa.gov). We calculated mean air temperature in May from 1979 – 2009 for each site as an index of each population's home

climate just before the nesting season. While the 30-year mean air temperature in May generally decreased with latitude (Supplementary figure 4), the NE site was cooler than the IA site despite being farther south. Therefore, we used the 30-year mean May temperature, rather than latitude, when regressing experimental parameters against home site (see section 3).

For each nest, we designated oviposition as day 0 and defined the incubation period as days 1 – 60. We defined the thermosensitive period (the middle third of embryonic development during which sex differentiation occurs in species with TSD) as days 21 – 40. Using the hourly temperatures recorded by data loggers installed at the time of oviposition, we described the incubation regime of each nest using four parameters. We determined both mean temperature and mean daily thermal range (i.e., largest – smallest temperature recorded during each 24-hour period) throughout the entire 60-day incubation period, and separately for the 20-day thermosensitive period.

We performed all statistical analyses using SAS 9.2 (SAS Institute, Cary, North Carolina). We analysed among-population differences in mean nesting date (i.e., phenology, with nest date defined as day of year such that day 1 = 1 January) and per cent shade cover over nests using Proc MIXED with female identity nested within population as a random effect; we analysed nest depth similarly except that we included female size as a covariate. Importantly, because females in some populations nested multiple times while others nested only once, we analysed nesting phenology using only the first nest constructed by each female. We included all nests in other analyses, again with female identity nested within population as a random effect. We compared among-population differences in nest sex ratios using a chi-square goodness-of-fit test (Wilson and Hardy, 2002). We employed Proc

GENMOD to model nest sex ratio (i.e., proportion male) and hatching success (i.e., proportion of eggs that hatched) with nest date, shade cover, nest depth, population, and all two-way interactions as possible predictors, and ranked candidate models using Akaike's Information Criterion corrected for small sample size (AIC_c). We considered the best-supported model and all models with $\Delta AIC_c < 2.0$ to be competing models.

2. Results

A total of 34 nests in the common-garden experiment were constructed by 26 females (4 from NM, 8 from IL, 3 from NE, 5 from IA, and 6 from WA). An unidentified IL female constructed one nest. Two IA females and four IL females nested twice, and one IA female nested three times. All females from NM, NE, and WA nested once. Despite these small sample sizes, however, robust differences among populations were observed. Female plastron length (a proxy for body size) was strongly positively correlated with both mean rear limb length ($r = 0.77$, $P < 0.0001$; Supplementary figure 5) and home climate ($r = 0.84$, $P < 0.0001$). Clutch size also increased as home climate became cooler ($r = -0.68$, $P = 0.001$) but populations did not differ in clutch size when controlling for female size (ANCOVA with plastron length as a covariate; $F_{4,33} = 0.90$, $P = 0.51$).

The date on which females constructed their first nest differed among the five populations, with IA and IL females nesting earlier than NM, NE, and WA females ($F_{4,21} = 4.11$, $P = 0.01$). Populations did not differ in the amount of shade cover over sites selected by females for nesting (mean for all populations = 25.9 ± 12.7%; $F_{4,33} = 0.83$, $P = 0.55$; Table 1; Fig. 1), and there was no significant correlation between shade cover and mean home climate ($R^2 = 0.016$, $P = 0.48$). Nest depth was positively correlated with female rear

limb length ($r = 0.62$, $P < 0.0001$) and, as female size increased with home climate, nest depth also increased with home climate ($r = 0.37$, $P = 0.03$). There was no evidence for among-population differences in mean nest depth when statistically controlling for female size ($F_{4,33} = 0.10$, $P = 0.98$; Fig. 2); thus, differences in nest depth among populations may have been caused by among-population differences in female size. Nest depth was inversely correlated with mean home climate ($r = -0.40$, $P = 0.02$), with females from warmer climates constructing shallower nests than females from cooler climates. Populations did not differ in any of the four incubation regime parameters (one-way analysis of variance; all P's > 0.20; Table 2). Hatching success differed among populations, with nests from the two coolest climates, NE and WA, exhibiting a lower proportion of surviving hatchlings than the other three populations (NE: 42.9 ± 51.5; WA: 42.7 ± 29.3; NM: 80.4 ± 13.2; IA: 84.0 ± 12.8; IL: 76.7 ± 19.8; $\chi^2 = 30.0$, $P < 0.0001$).

We observed one intersex hatchling, which emerged from a WA nest and possessed one ovary and one testis. NE was excluded from analyses of sex ratio due to low nest survival; however, the single surviving NE nest yielded a sex ratio of 0.5 and, along with a nest from NM, had the smallest proportion of male hatchlings in this study. Excluding NE, the sex ratio produced by females did not differ among populations ($\chi^2 = 2.49$, $P = 0.65$; Fig. 3). Including NE, the nest-site choice model for predicting nest sex ratio with the lowest AIC_c included the parameters Population and Nesting date; the model containing Nesting date and Shade cover was within 2 ΔAIC_c. No other models were competitive.

3. Discussion

Adaptation to climate change may be impossible even when high genetic variation is present if the rate of environmental change is too rapid and the population demography is insufficiently dynamic (reviewed in Lavergne et al., 2010). The current, rapid rate of global climate change may preclude long-lived organisms, in particular, from keeping pace through adaptive cross-generational genetic changes because the lag between environmental conditions and optimal trait values may lead to strong directional selection that quickly decreases genetic variation and leads to extinction (reviewed in Hoffman and Sgrò, 2011). Instead of rapid genetic adaptation to climate change, mechanisms such as genetically-based phenotypic plasticity and/or non-genetic forms of plasticity may be necessary. In our study, females from transplanted populations showed similar choice of shade cover over nests to local females, which suggests that behavioural phenotypic plasticity in female choice of shade cover over the nest site may comprise an immediate mechanism by which long-lived reptiles with TSD can avoid skews in sex ratio potentially caused by rapid climate change. Indeed, we hypothesize that phenotypic plasticity should be a general mechanism by which organisms with long generation times respond immediately to accommodate rapid changes in phenotypic selection (sensu Price et al., 2003). Global temperatures are predicted to increase by 1.8 – 6.4^{o}C over the 21st century (Solomon et al., 2007), and an increase of this magnitude is likely to exceed the adaptive potential of a relatively long-lived species such as *C. picta* (Hoffmann and Sgrò, 2011, but see Rosenheim and Tabashnik, 1991).

We found that populations from across the geographic range of *C. picta* differed in nesting date and nest depth despite experimental exposure to a common environment. Nesting phenology is likely constrained by the number of clutches produced per year, which

varies in this species: populations in which females nest multiple times in a season likely have less plasticity in nesting date than populations where females nest only once in a season. Indeed, in this study, the populations that constructed their first nests earlier were also those that nested multiple times; populations that nested later also nested once. Importantly, there was no relationship between the length of time spent in the common garden and nesting date: both NM and WA females, who spent the most and the least time, respectively, in the common garden, nested late in the season. IA and IL females, whose time in the common garden was intermediate to NM and WA females, nested significantly earlier than either of those populations. Interestingly, while climate change may increase the length of the nesting season and thereby allow some populations that currently nest twice to nest a third time in a single season, sex ratio skew may paradoxically increase because the thermosensitive period of the third nest is likely to occur during cooler conditions (Schwanz and Janzen, 2008). Regardless of its effects on sex ratio, however, timing of nesting has low heritability in *C. picta* (McGaugh et al., 2010) and therefore shifts in nesting phenology are likely to be behaviourally plastic rather than inherited (Schwanz and Janzen, 2008).

Nest depth affects the incubation conditions experienced by developing embryos (J.M.R., unpublished data), and thereby may affect nest sex ratio. Reptiles that construct subterranean nests by digging with their front limbs, such as lizards and tuatara, can vary nest depth by simply digging deeper (Nelson et al., 2004). Freshwater turtles, however, construct nests using their back limbs, which suggests that maximum nest depth in turtles is constrained by rear limb length. In our study, females from southern populations were smaller and dug shallower nests than females from more northern populations. In their home environment, however, NM females dug deeper nests than those from IL (Morjan, 2003a),

suggesting that NM populations are currently constructing nests of maximum depth for their body size, and likely have less adaptive potential to shift nest depth than populations in which mean nest depth is currently shallower than the maximum depth possible based on female size. While increased adult mortality can cause rapid shifts in growth rate and adult size in reptiles (e.g., Wolak et al., 2010), indirect selection for increased female body size for deeper nest construction is likely much weaker. Therefore, increases in female body size will probably occur only gradually, and compensation for rapid climate change through selection for deeper nests is likely to be evolutionarily constrained by relatively weak selection for increased female size.

Populations in the current common-garden experiment did not differ in female choice of shade cover over nests, despite all females having shade cover ranging up to 70% from which to choose a nest site, and this lack of difference in choice of shade cover resulted in similar incubation regimes among populations. Shade cover is a reliable predictor of nest sex ratio in the IL population (Janzen, 1994b), and females may adjust choice of nest shade cover in order to match a nest site's future incubation regime to prevailing environmental conditions (e.g., Doody et al., 2006a). Importantly, however, the ability to adjust choice of shade cover can only be expressed if a range of shade cover options is available. For example, a wider range of shade cover is available at nesting areas used by the IL population compared to the NM population (J.M.R., unpublished data), so it is possible that less plasticity in choice of shade cover exists for the NM population. Nevertheless, the lack of among-population difference in nest shade cover observed in our study suggests that females from a range of local climates adjusted their choice of nest shade cover in response to environmental conditions in the common-garden experiment.

In a common-garden experimental design, one possible explanation for a lack of observed difference in a trait among populations is that the trait is not phenotypically plastic among populations. Therefore, the similarity among populations in choice of shade cover over nests observed in our study could be reflective of a lack of plasticity in this trait. However, data on nest-site choice at the home sites of some of the study populations support our contention that choice of shade cover over nests was adjusted in response to the common-garden conditions. In particular, in the common-garden experiment females from IL and WA chose sites with less shade cover, and those from NM chose approximately the same amount of shade cover, as sites chosen by females in these populations at their home sites (Morjan, 2003b; McGaugh et al., 2010; J.M.R. and F.J.J., unpublished data). These differences in choice of shade cover between the common-garden and home site conditions suggest that females in the common-garden experiment were adjusting their choice of shade cover in response to environmental conditions at the experimental site. Moreover, the selection of similar amounts of nest shade cover among females in this study resulted in similar incubation conditions and similar nest sex ratios across populations. These results suggest that choice of nest shade cover is a behaviourally plastic mechanism by which female turtles can influence the sex ratio of their offspring. Importantly, however, a diversity of shade cover must be available to nesting turtles in order for this plasticity to be expressed. Behavioural plasticity in traits such as maternal choice of nest shade cover, which can be adjusted based on immediate environmental conditions, may be important mechanisms in allowing species with long generation times to adjust to rapid climate change when adaptive changes in allele frequencies occur too slowly to compensate.

Acknowledgments

Funding for this study came from the Iowa Academy of Sciences, Society for Integrative and Comparative Biology, Society for the Study of Amphibians and Reptiles and the Dean Metter Memorial Fund, Sigma Xi, and Iowa State University's Department of Ecology, Evolution, and Organismal Biology (all to J.M.R.), while F.J.J. received support from NSF DEB-0640932. Thanks to J. Clapp and M. Fritz for collecting permits, and C. Lee for hospitality at Bosque del Apache NWR. R. Alverio-Newton, K. Christensen, R. Clayton, E. Holman, N. Howell, J. Kubik, T. Mitchell, J. Stuart, H. Streby, J. Strickland, and D. Warner helped with animal collection and maintenance of the experimental ponds. This manuscript was much improved by valuable comments from A. Bronikowski, P. Dixon, C. Kelly, E. Takle, H. Streby, and three anonymous reviewers. This research was conducted in accordance with Institutional Animal Care and Use Committee protocol #1-09-6677-J (Iowa State University).

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Table 1. Population means ± standard deviation of nest-site choice parameters and nest sex ratio for *Chrysemys picta bellii* collected from 5 populations (listed in order of decreasing latitude) that nested in a semi-natural common-garden experiment in Story Co., IA in 2009.

Population	Number of nests	Nesting date	% shade cover	Nest depth (mm)	Nest sex ratio (proportion male)
WA	6	20 June ± 6.1	19.0 ± 6.6	103.3 ± 24.0	0.87 ± 0.18
IA	8	12 June ± 11.0	26.9 ± 14.6	96.2 ± 4.4	0.92 ± 0.16
NE	3	20 June ± 7.0	35.0 ± 21.1	91.7 ± 12.6	--*
IL	13	6 June ± 7.8	25.8 ± 10.1	89.2 ± 9.7	0.89 ± 0.12
NM	4	20 June ± 2.6	27.8 ± 17.5	83.8 ± 11.1	0.88 ± 0.25

* Sex ratio was not calculated for NE due to low nest survival

Table 2. Population means of nest incubation regime parameters of *Chrysemys picta bellii* collected from five populations that nested in a common-garden in Story Co., IA in 2009. All values are mean ^{o}C ± standard deviation. Thermosensitive period (TSP) was considered days 21 – 40 of the 60-day incubation period.

	WA (n = 2)	IA (n = 3)	NE (n = 2)	IL (n = 6)	NM (n = 3)
incubation period temp	23.54 ± 0.09	23.05 ± 0.34	22.96 ± 0.63	23.65 ± 0.63	23.65 ± 0.36
TSP temp	23.06 ± 0.02	22.51 ± 0.63	22.63 ± 0.54	23.60 ± 0.83	23.09 ± 0.21
Daily temp range, incubation period	7.32 ± 0.08	6.02 ± 0.36	6.07 ± 0.78	7.02 ± 2.35	7.38 ± 0.76
Daily temp range, TSP period	7.76 ± 0.06	6.03 ± 0.52	6.44 ± 0.94	7.53 ± 2.06	7.60 ± 0.60

Figure legends

Figure 1. Percent shade cover over nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Populations did not differ in % shade cover ($F_{4,33} = 0.83$, $P = 0.55$).

Figure 2. Depth of nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Nest depth was positively correlated with female rear limb length ($r = 0.62$, $P < 0.0001$) and increased with latitude of home population ($r = 0.37$, $P = 0.03$). Closed circle = NM, open circle = IL, triangle = NE, square = IA, diamond = WA.

Figure 3. Sex ratios of nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Sex ratio did not differ among populations (NE excluded due to low nest survival; $\chi^2 = 2.49$, $P = 0.65$).

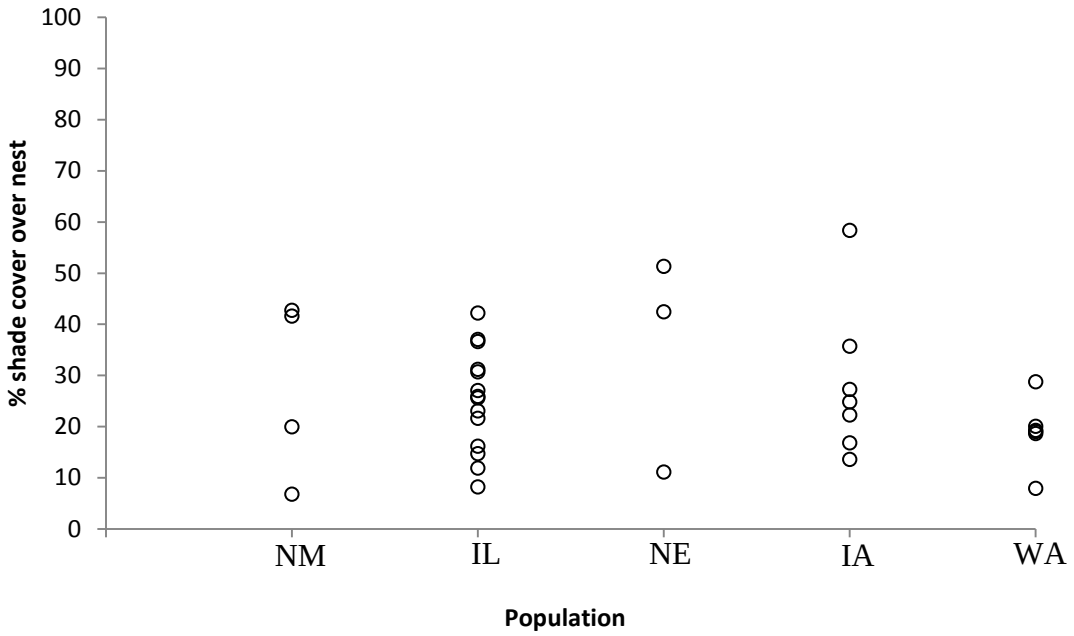

100
90
80
70
60
50
40
30
20
10
0
% shade cover over nest
NM
IL
NE
IA
WA
Population

Figure 1

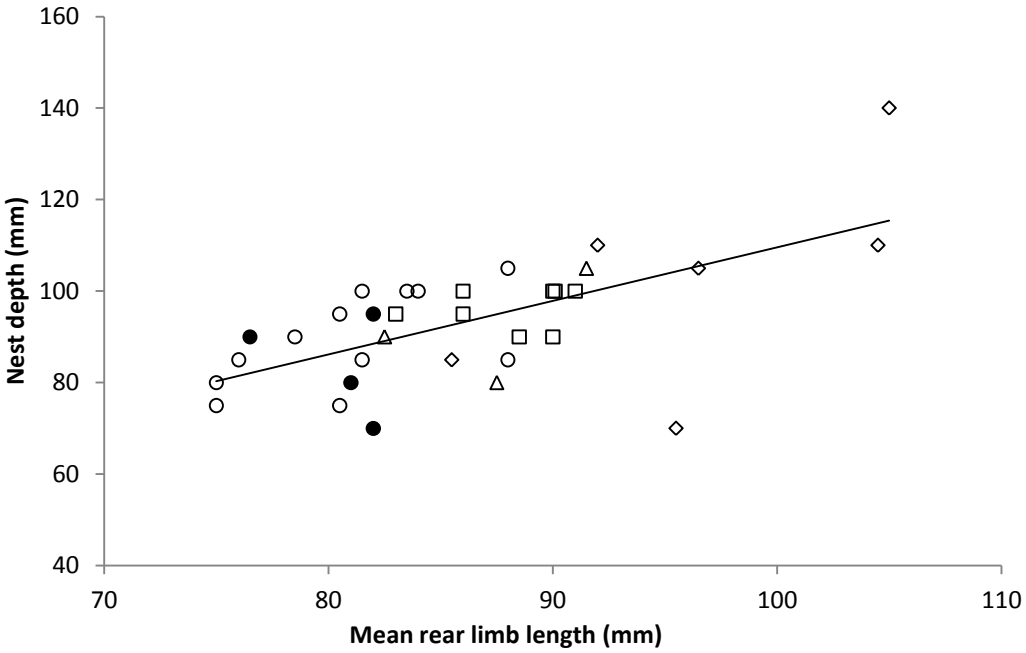

160
140
120
100
80
60
40
Nest depth (mm)
70
80
90
100
110
Mean rear limb length (mm)

Figure 2

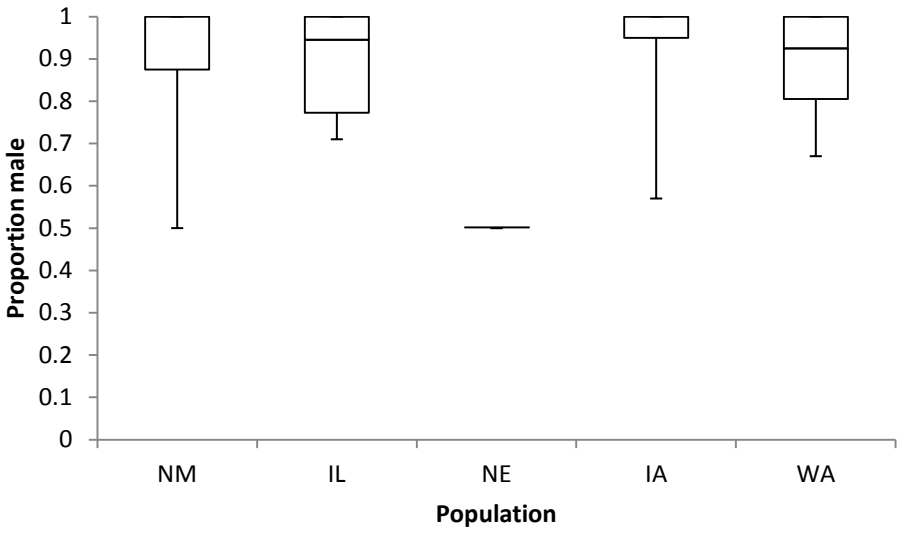

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion male
NM
IL
NE
IA
WA
Population

Figure 3

Supplementary data – color figures are for Web publication only

Supplementary figure 1. Range of the painted turtle, *Chrysemys picta* (from Ernst, C.H., Lovich, J.E., 2009. Turtles of the United States and Canada, second edition. Johns Hopkins University Press, Baltimore.) Outline encloses the range of the western subspecies, *C.p. bellii*. Black circles indicate locations from which turtles were collected for use in this experiment (Bosque del Apache National Wildlife Refuge, Socorro County, NM; Thomson Causeway Recreation Area, Carroll County, IL; Hyannis, Grant County, NE; Ames, Story County, IA; and Pierce National Wildlife Refuge, Skamania County, WA).

Supplementary figure 2. Series of experimental ponds in which *Chrysemys picta bellii* were housed at Iowa State University's Aquatic Research Facility, Story County, IA. The pond in the foreground is the western-most pond, with ponds situated in parallel from west to east and oriented with the long axis of each pond running from north (foreground) to south. Ponds are 15 m (west-east axis) by 40 m (north-south axis). Note the drift fencing surrounding all ponds to prevent escape of experimental turtles and entry of local turtles. Artificial shade objects at each pond are not shown.

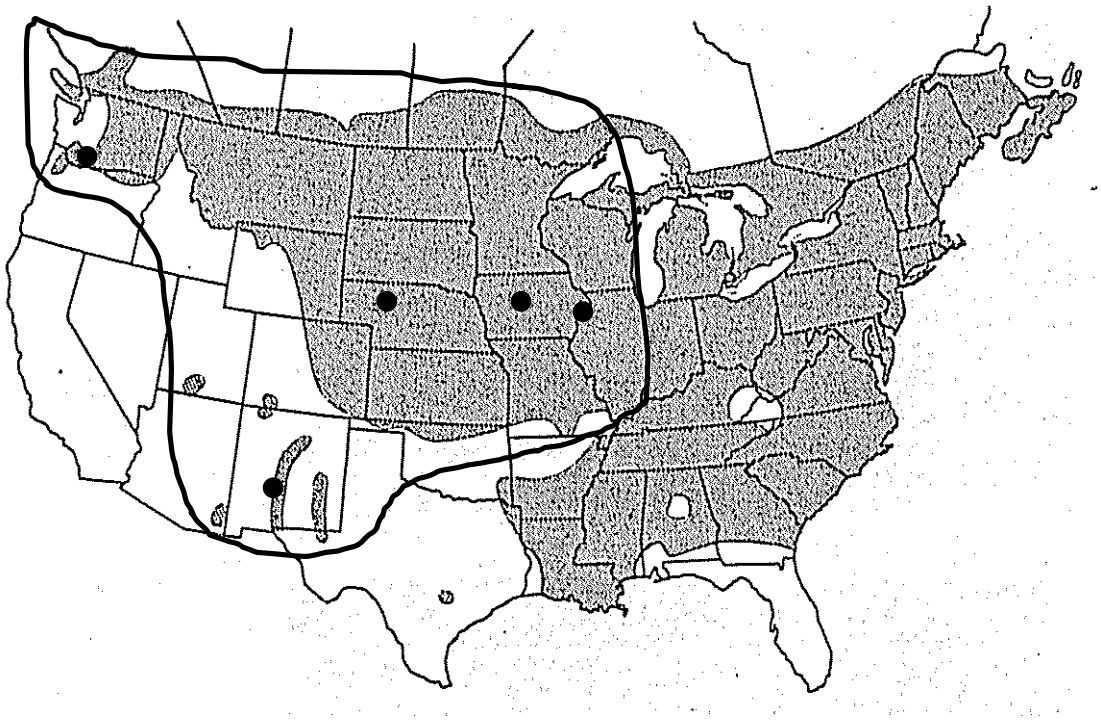
Supplementary figure 3. A female painted turtle, *Chrysemys picta bellii*, nesting in the outdoor common-garden experiment at Iowa State University's Aquatic Research Facility, Story County, IA. This female is in the process of arranging eggs within the nest cavity with her left rear limb.

Supplementary figure 4. Mean air temperature in May from 1979 – 2009 at each population's site of collection. Climate data are from the National Climate Data Center (www.ncdc.noaa.gov) and were collected at Bosque del Apache (NM site), Clinton, IA (IL site), Hyannis, NE (NE site), Ames 8 WSW (IA site), and Skamania Fish Hatchery (WA

site). Data were unavailable for some sites in some years. Populations are listed in the legend in order of increasing latitude.

Supplementary figure 5. Population means of female size and nest characteristics of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. All values are means ± standard deviation.

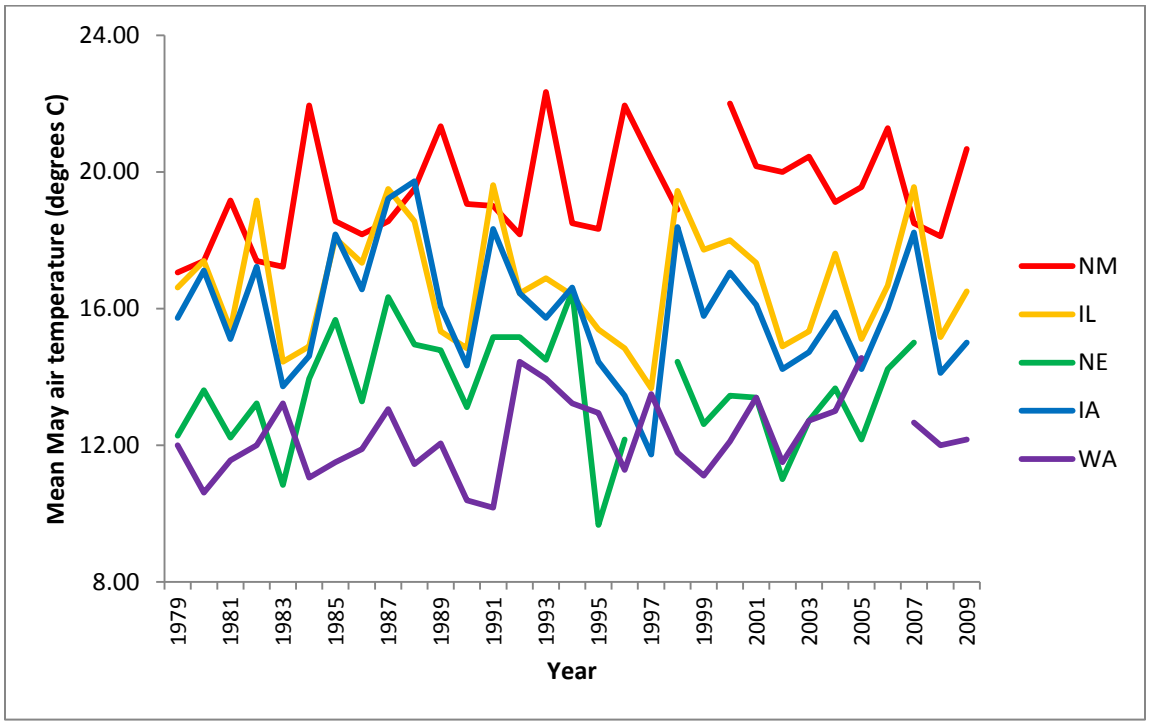
Supplementary figure 1



Supplementary figure 2

Supplementary figure 3

Supplementary figure 4



Supplementary figure 5

Population	Number of females	Female rear limb length (mm)	Female plastron length (mm)	Clutch size	Egg mass (g)
NM	11	80.4 ± 2.6	138.5 ± 3.7	7.5 ± 1.7	6.42 ± 0.68
IL	15	80.9 ± 4.3	155.8 ± 4.5	9.9 ± 1.6	6.03 ± 0.84
NE	6	87.2 ± 4.5	153.7 ± 4.0	11.3 ± 2.1	5.84 ± 0.76
IA	15	87.7 ± 3.2	165.4 ± 8.4	11.4 ± 2.1	6.99 ± 0.71
WA	13	96.5 ± 7.5	173.7 ± 13.0	13.5 ± 3.0	6.42 ± 0.73

CHAPTER 4. HIGH THERMAL VARIANCE IN NATURALLY-INCUBATED TURTLE NESTS PRODUCES FASTER OFFSPRING

A paper submitted for publication to *Functional Ecology*

Jeanine M. Refsnider

Abstract

The effects of climate change on populations are complex and difficult to predict, and can result in mismatches between interdependent organisms or between organisms and abiotic factors. Reptiles with temperature-dependent sex determination may be able to compensate for potential skews in offspring sex ratio caused by climate change by selecting cooler (i.e., shadier) nest sites. However, although changing nest location may prevent sex ratio skews, it may also affect thermally-sensitive performance traits in offspring. I tested righting, sprinting, and swimming performance in hatchling painted turtles (*Chrysemys picta*) produced by female turtles from five populations across the species' geographic range nesting in a common-garden environment. I found that speed of hatchling performance tended to increase with increasing mean temperature of the mother's population of origin, and that nests with higher mean daily variation in incubation temperature produced faster hatchlings. These results suggest that the increased temperature fluctuations predicted by climate change models could result in hatchling turtles that are faster at righting themselves, sprinting, and swimming; however, it is not yet known how these performance measures translate into fitness.

Introduction

How organisms and communities will respond to climate change is a burgeoning question in biology. Already, shifts in species' ranges and phenological timing concurrent with recent climate change have been documented in numerous taxa (e.g., Bradley et al. 1999; Parmesan et al. 1999). Importantly, however, the effects of such changes across taxa are highly inconsistent and difficult to predict. For example, although range shifts may allow a species to track its climatic envelope poleward as global climate changes, such a shift will bring organisms into contact with new individuals and could result in decreased fitness through competitive exclusion (Atkins & Travis 2010), agonistic encounters (Jankowski, Robinson & Levey 2010), and heightened disease risk (Kutz et al. 2005). Similarly, although some species have advanced their reproductive activities to correspond with the earlier occurrence of spring (e.g., Crick et al. 1997; Nussey et al. 2005), this alteration of phenology may be maladaptive if it results in a mismatch between interdependent species (Visser & Both 2005) or in poor timing decisions based on global cues that are out of synchrony with current local conditions (Inouye et al. 2000).

Understanding the mechanism(s) underlying species' responses to climate change may improve our ability to predict those responses. Contemporary climate change has caused rapid adaptive genetic change in some short-lived organisms (e.g., Bradshaw & Holzapfel 2001; Umina et al. 2005; Balanyá et al. 2006; Pen et al. 2010) and, indeed, micro-evolutionary processes may allow the persistence of some taxa through periods of climate change. However, the rapid rate at which contemporary climate change is occurring compared to past episodes of climate change may preclude organisms with longer generation times from adapting to climate change via changes in allele frequencies. Instead, long-lived

species may rely on non-genetically-based mechanisms such as behavioural plasticity to acclimatize to a different climate, at least until allele frequencies can "catch up" (e.g., Beebee 1995; Berteaux et al. 2004).

Many long-lived reptile groups have temperature-dependent sex determination (TSD), whereby offspring sex is irreversibly determined by the temperature experienced by developing embryos. The extreme thermal sensitivity of this sex-determining mechanism means that a small change in environmental temperature could dramatically alter offspring sex ratio; thus, anthropogenically-driven climate change has the potential to create populations of reptiles with TSD that consist of predominantly one sex (Janzen 1994; Nelson et al. 2004; Wapstra et al. 2009; Schwanz et al. 2010). Reptiles with TSD have survived periods of past tumultuous climate change, and their persistence despite numerous periods of global warming and cooling has been attributed to two potential mechanisms (Bulmer & Bull 1982): 1) changes in the specific incubation temperatures producing each sex, or 2) changes in maternal nest-site choice, which affects the microclimate experienced by developing embryos. Although both traits are likely to have an influence over evolutionary timescales, heritability of thermal sensitivity in sex determination appears insufficient to compensate for skewed sex ratios resulting from rapid climate change (Janzen 1994), and low phenotypic variation between populations suggests that the sex-ratio reaction norm is an insufficient target for selection to compensate for climatic differences among populations (Morjan 2003a; but see McGaugh & Janzen 2011). Moreover, micro-evolutionary responses of maternal nest-site choice to a rapidly changing climate also appear to be insufficient to compensate for rapid climate change (Morjan 2003b*;* McGaugh et al. 2010). Therefore, if long-lived reptiles with TSD are to avoid skewed sex ratios produced by temperatures changing at

unprecedented rates, behavioural phenotypic plasticity in nest-site choice may be the likely immediate mechanism (Morjan 2003b).

In a common-garden experiment on a turtle with TSD, I found that females from five populations across the species' geographic range chose nest sites with similar amounts of shade cover, which produced clutches of similar sex ratios (Refsnider & Janzen in press). Thus, females appeared to compensate for the effects of novel climatic conditions on offspring sex ratio via behavioural plasticity in nest-site choice. Importantly, however, the nest site and its resultant incubation regime affect numerous offspring traits in addition to sex ratio, including size (Brown & Shine 2004), growth rate (Brooks et al. 1991), metabolism (Van Damme et al. 1992), speed (Miller 1993), and predator avoidance behaviour (Burger 1989). Maternal selection of oviposition site likely operates hierarchically, with sites selected to maximize maternal or offspring survival first, and hatchling phenotype secondarily (Refsnider & Janzen 2010). Therefore, it is likely that shifts in maternal nesting behaviour in response to climate change, which function primarily to maximize offspring survival and/or to influence sex ratio, will have simultaneous and perhaps unexpected effects on offspring size and performance.

To determine whether the observed shift in nesting behaviour of turtles exposed to novel environmental conditions affected offspring phenotypes, I measured the size and performance of offspring produced by female turtles from different populations nesting in the common-garden environment from Refsnider & Janzen (in press). In several turtle species, larger hatchlings are more likely to survive migration from the nest to a wetland (Janzen 1993; Janzen, Tucker & Paukstis 2000; Paitz et al. 2007); therefore, I considered size (carapace length and body mass) to be an indicator of hatchling quality. Incubation

conditions also affect hatchling turtles' running (Miller, Packard & Packard 1987; Janzen 1995) and swimming speed (Miller 1993), as well as ability to right themselves when placed on their carapace (Freedberg, Ewert & Nelson 2001; Colbert, Spencer & Janzen 2010). Righting speed is thought to be an indicator of muscular coordination, physical strength, and predator-escape ability (Burger 1976; Freedberg et al. 2004; Delmas et al. 2007). Therefore, nest microhabitats that produce hatchlings that are large, fast, and/or have enhanced righting ability may be favored by females over microhabitats that produce smaller and/or slower hatchlings. I tested the null hypothesis that, because turtles from different populations chose similar nest sites which resulted in similar incubation regimes and thereby similar offspring sex ratios (Refsnider & Janzen in press), offspring would be similar in size and performance despite differing in provenance. Rejection of the null hypothesis would indicate that, despite compensating for novel climatic conditions by preventing sex-ratio bias, behavioural plasticity in maternal nest-site choice affected offspring phenotype in other important ways. My secondary objectives were to determine which parameters of nest-site choice and incubation regime best predict offspring size and performance.

Materials and methods

I used the western painted turtle, *Chrysemys picta bellii*, as a model species in this experiment. Painted turtles are widely distributed in the U.S. and southern Canada, where they inhabit a variety of freshwater habitats. Females nest in open areas such as beaches or lawns in early summer, and incubation lasts ~60 d. After hatching, neonates remain in the nest cavity through their first winter and emerge the spring following nest construction, at which time they travel terrestrially until reaching a wetland habitat. Predation on nests

ranges from 19 – 95% (Strickland & Janzen 2010), and survival of migrating hatchlings is around 60% (with losses primarily attributed to predation; Paitz et al. 2007). This species has temperature-dependent sex determination, with females produced at constant incubation temperatures above 29^{o}C and males produced at constant temperatures below 27^{o}C (Ewert, Jackson & Nelson 1994).

Animal collection and housing

Collection and housing of adult turtles is described in detail in Refsnider & Janzen (in press). Briefly, gravid female were collected from Socorro Co., New Mexico; Carroll Co., Illinois; Grant Co., Nebraska; Story Co., Iowa; and Skamania Co., Washington in spring 2009. Turtles were housed in identical experimental ponds in an outdoor common garden in Story Co., Iowa, where they had free access to nesting habitat. I monitored the experimental enclosure for nesting activity from 27 May – 3 July 2009. Turtles observed nesting were quickly identified, and were then monitored from a distance to prevent nest abandonment due to disturbance. I excavated all nests within 24 h of construction to determine clutch size and measure nest depth; I also recorded the date of construction, took a hemispherical photograph over the nest site to quantify shade cover using Gap Light Analysis software (Frazer, Canham & Lertzman 1999), and inserted a data logger (iButtons, Embedded Data Systems, Kentucky, USA) amongst the eggs to record nest temperature hourly throughout incubation. Nests then remained in situ for the duration of the incubation period.

In October 2009 (after hatching but before emergence from the nest), I excavated all nests to retrieve hatchlings and data loggers. I housed clutch-mates together in plastic deli cups containing moist soil. Hatchlings were carefully cleaned and dried, and the plastron of each was scanned to facilitate individual identification. All hatchlings were weighed and

measured (straight carapace length; Demuth 2001), and then over-wintered in the same covered deli cups at 4^{o}C in a temperature-controlled incubator (Revco, Thermo Scientific, North Carolina, USA). Starting in mid-March 2010, incubator temperature was gradually increased to 19^{o}C over a 2-week period. Following this acclimatization period, hatchlings were housed at 19^{o}C.

Performance tests

I conducted three performance tests on each hatchling: 1) righting time (the time it took a hatchling placed on its back to right itself); 2) sprinting time (the time it took a hatchling to walk 0.5 m); and 3) swimming time (the time it took a hatchling to swim 1.0 m). These performance tests simulated a hatchling's journey from the nest site to a wetland habitat, and then to suitable habitat within the wetland, following nest emergence in the spring. Therefore, I tested each hatchling in the order of righting time, immediately followed by sprinting time, followed immediately by swimming time. Each hatchling underwent two performance trials of the three performance tests, with trials approximately two weeks apart.

The righting time test was conducted by placing a hatchling on its carapace on a circular piece of Astroturf, and recording the time taken by the hatchling to flip itself right side up. Sprinting and swimming tests were conducted with the hatchling placed at one end of a metal trough; I lightly tapped on the posterior of the carapace with a pencil to encourage movement. The surface of the sprinting track was lined with Astroturf, and the swimming track was filled with 5 cm of water at 19^{o}C. In all three performance tests, I timed a hatchling's latency to begin moving (Latency), the total time taken to complete the test from initial placement of the hatchling until the test was completed (Total), and the time during which the hatchling was actively moving during completion of the test (Active, or Total –

Latency). All times were recorded to the nearest second using a digital stopwatch, and all tests were censored at 180 s.

Statistical analyses

I acquired climate data for each collecting site from the National Climate Data Center (www.ncdc.noaa.gov). I calculated mean May air temperature from 1979 – 2009 for each site as an index of each population's home climate at the onset of the nesting season.

For each nest, I designated oviposition as day 0 and defined the incubation period as days 1 – 60. I defined the thermosensitive period (the middle third of embryonic development during which sex differentiation occurs in species with TSD) as days 21 – 40. Using the hourly temperatures recorded by data loggers installed at the time of oviposition, I described the incubation regime of each nest using four parameters. I determined both mean temperature and mean daily thermal range (i.e., largest – smallest temperature recorded during each 24-hour period) throughout the entire 60-day incubation period, and separately for the 20-day thermosensitive period.

All statistical analyses were conducted using SAS 9.2 (SAS Institute, North Carolina, USA). I analysed among-population differences in mean hatchling mass and carapace length using one-way analysis of variance in the MIXED Procedure with maternal identity as a random effect. Population and sex differences in hatchling performance (i.e., Latency, Active, and Total median times for righting, sprinting, and swimming) were analysed using Kruskal-Wallis tests in the NPAR1WAY procedure (as censored data required use of rank-based tests; see below). Sexes did not differ in any performance measure (all P values > 0.1); therefore, sex was not included in further analyses. Among-population variation in mean hatchling mass and carapace length was evaluated using the MIXED procedure with

population, nest date, nest depth, and shade cover as independent predictors; maternal identity nested within population as a random effect; and maternal plastron length as a covariate. The same procedure was also used to evaluate the effect of incubation regime on mean hatchling mass and carapace length; in these models, population, mean incubation temperature, mean thermosensitive period temperature, mean daily thermal range throughout incubation, and mean daily thermal range during the thermosensitive period were independent predictors; maternal identity nested within population was a random effect; and maternal plastron length was a covariate. Candidate models, including null models, were ranked using Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham & Anderson 2002). I considered the best-supported model and all models with $\Delta AIC_c < 2.0$ to be competing models.

I analysed the effects of nest-site choice and incubation regime on each measure of hatchling performance (i.e., Latency, Active, and Total times for righting, sprinting, and swimming) using tobit regression with all performance measures censored at 180 s. This approach allowed me to include hatchlings that failed to perform during any 180-second performance test, as non-performance is likely to be biologically significant (e.g., Janzen 1995). Importantly, however, because the random effect of hatchlings nested within females on the scale of absolute performance time is not equivalent to the same random effect on the rank scale, I used as an approximation the median performance time within each clutch, rather than individual hatchlings' performance times nested within a clutch, as the experimental unit. Although this approach ignores within-hatchling differences in performance, it is equivalent to taking the median of each hatchling's performance measures and then the median of all hatchlings within a female, which allowed me to use the

nonparametric tobit regression analysis described above. I found no significant correlation of hatchling performance residuals within females (all P values > 0.30), indicating no significant differences in hatchling performances among females after accounting for nest-site choice and incubation regime variables.

As independent predictors of hatchling performance, I used population, nest date, nest depth, and shade cover (nest-site choice model) or population, mean incubation temperature, mean thermosensitive period temperature, mean daily thermal range throughout incubation, and mean daily thermal range during the thermosensitive period (incubation regime model). Because I left eggs to incubate and hatch in situ, it was not possible to match eggs with hatchlings and therefore I could not include egg mass as a covariate in these analyses; however, including maternal identity as a random effect accounts for this variance component. All P-values were corrected for multiple comparisons (m = 9) using the Bonferroni adjustment.

Results

A total of 203 surviving hatchlings was produced from 34 nests constructed by 26 individual females (four from NM females, eight from IL, three from NE, five from IA, and six from WA) in the common-garden experiment. Several females from the IA and IL populations nested two or three times; all other females nested once. Hatchlings produced by females from different populations did not differ in mass ($F_{4,174}$ = 0.78, P = 0.542) or carapace length ($F_{4,174}$ = 0.52, P = 0.72; Table 1). Hatchling mass and carapace length were not significantly correlated with mean May home temperature (all P's > 0.20).

Median performance times for active sprint time (χ^2 = 20.66, P < 0.01), total sprint time (χ^2 = 19.95, P = 0.009), latency to swim (χ^2 = 19.68, P < 0.01), and total swim time (χ^2 = 18.47, P = 0.009) differed among populations (Fig. 1). Moreover, these performance measures tended to increase with increasing mean May temperature at the mother's site of origin; that is, hatchlings from cooler home climates tended to be slower at both sprinting (active sprint time: R^2 = 0.06, P = 0.009) and swimming (total swim time: R^2 = 0.06, P = 0.09) than hatchlings from warmer home climates (Fig. 1). No measures of righting time differed among populations (all P values > 0.2). Hatchling mass was not correlated with any performance measure (all P values > 0.2), but hatchlings with longer carapaces had shorter median active sprint times ($F_{1, 303}$ = 8.27, P = 0.036, R^2 = 0.027).

The nest-site choice model for predicting hatchling mass with the lowest AIC_c included the parameters Population, Nest date, and Shade cover; the best nest-site choice model for predicting hatchling carapace length included only Population and Nest date. No other models were competitive. The best incubation regime models for predicting both hatchling mass and carapace length were the full models; for hatchling mass, the model containing all parameters except Mean daily thermal range throughout incubation was within 2 ΔAIC_c of the full model. The only nest-site choice variable that significantly predicted hatchling performance was Shade cover, which was negatively correlated with sprinting time (active sprint time: t_1 = -2.71; P = 0.007; total sprint time: t_1 = -2.16; P = 0.03).

Populations did not differ in any of the four incubation regime parameters (one-way analysis of variance; all P's > 0.20; see Table 2 in Refsnider & Janzen in press). However, several incubation regime parameters were significant predictors of hatchling performance. Nests with greater mean daily thermal range during the thermosensitive period produced

hatchlings that were faster at both righting themselves (latency to right: $t_1 = 2.80$, $P = 0.045$; active righting time: $t_1 = 3.46$, $P < 0.01$) and swimming (total swimming time: $t_1 = 3.84$, $P < 0.01$) than nests with lower mean daily thermal range during the thermosensitive period (Fig. 2). Nests with higher mean temperatures during the thermosensitive period also produced hatchlings with a shorter latency to swim ($t_1 = -2.88$, $P = 0.036$; Fig. 3).

Discussion

I found that hatchling painted turtles produced in a common-garden environment tended to be slower and more reluctant to perform when their mothers originated from a cooler home climate. Further, nests with higher mean daily thermal range during both the thermosensitive period and throughout the entire incubation period were associated with faster hatchlings that performed more readily than nests with lower mean daily thermal range.

Most other studies on turtles that found an effect of incubation temperature on hatchling performance have incubated eggs under controlled laboratory conditions at essentially constant temperatures (e.g., Janzen 1995; Freedberg et al. 2001; Freedberg et al. 2004; Colbert et al. 2010). A few studies have incorporated daily thermal fluctuation into laboratory incubation conditions, but the results have been inconsistent: greater thermal fluctuation resulted in faster swimming speeds in hatchling softshell turtles (Ashmore & Janzen 2003; but also see Mullins & Janzen 2006), but thermal fluctuation had no effect on righting time in painted turtles (Paitz et al. 2010). This study, which measured incubation temperatures in natural nests, showed that hatchling performance was positively correlated with mean daily thermal range during incubation. Because thermal variation, in addition to

mean temperature, also influences sex determination in reptiles with TSD (e.g., Georges, 1989; Warner & Shine 2011), it is not surprising that daily thermal range also affects the thermally-sensitive hatchling performance traits examined here. Moreover, previous studies on both turtles (Janzen 1995) and snakes (Burger 1991) have found that hatchling performance is differentially affected by intermediate vs. extreme constant incubation temperatures, and my results support the general influence of thermal variance on offspring performance. Generally, tests of performance in reptiles are conducted under a single thermal regime, which assumes that there is no interaction between incubation conditions and testing conditions on offspring performance. In addition to studying the effects of different incubation thermal regimes on offspring performance, it would therefore also be useful to know how such effects are manifested across different testing thermal regimes.

I found among-population differences in both sprinting and swimming performance by hatchling turtles produced in the common-garden experiment. Hatchlings whose mothers were from northern, cooler home climates were slower and more reluctant to both sprint and swim than hatchlings from southern, warmer maternal home climates. This result suggests that, although female turtles from transplanted populations appeared to prevent sex-ratio bias under the novel climatic conditions of the common-garden through selection of shade cover over the nest, similarities in nest-site choice did not have similar effects on offspring performance. How such differential hatchling performance occurs, however, is presently unknown: hatchling performance could be heritable, in which case wild populations in cooler climates would be expected to produce slower hatchlings than populations in warmer climates; alternatively, incubation conditions may differentially affect the performance of hatchlings from different populations.

It is important to note that, while incubation regime had a definite effect on hatchling performance in our study, the relationship between performance of hatchlings and reproductive fitness as adults in turtles is not well-understood. Studies on other taxa that also showed effects of incubation temperature on juvenile performance were similarly unable to tie performance of early life-stages to fitness of adults (e.g., fish: Rodríquez-Muñcoz, Nicieza, & Braña 2001, Baynes & Howell 1996; amphibians: Watkins & Vraspir 2006). Understanding how juvenile performance relates to fitness in adults would enhance our ability to predict phenotypes of later life stages from the performance of earlier ones.

In addition, we do not know precisely what constitutes "good" performance in the study species. Moreover, performance aspects that affect fitness likely differ among populations due to ecological factors such as the local complement of predators, the distance hatchlings must migrate from the nest to a wetland and the vegetation and topography they must travel through, and characteristics of the wetlands hatchlings eventually enter. Righting ability is important in diamondback terrapin hatchling ability to escape nest predation (Burger 1976), but poor righting ability at hatching does not carry through to survival during migration in painted turtles and therefore may not be ecologically relevant in this species (Colbert et al. 2010). Similarly, although hatchling map turtles with slower running speeds were associated with lower survival (Freedberg et al. 2001), in snapping turtles, hatchlings with lower propensity to run had higher survival than hatchlings with high propensity to run, and no significant selection on running performance was detected (Janzen 1995). It is possible that even a "fast" hatchling turtle is still too slow to evade capture by terrestrial predators, so righting ability and sprinting speed may not actually enhance ability to escape predation. Instead, behaviours such as freezing to escape the notice of predators (e.g., Janzen

1995) or high physiological endurance (particularly for populations that nest at greater distances from wetlands; e.g., Jayne & Bennett 1990; Kolbe & Janzen 2002) may more accurately predict fitness of hatchling turtles. Swimming speed may be a more useful measure of hatchling performance than righting or sprinting speed, because hatchling turtles must escape aquatic predators once they reach a wetland; the speed and maneuverability of hatchling turtles in water, as opposed to that on land, likely aids turtles in evasion of aquatic predators. Nevertheless, it is important to tie commonly-used measures of hatchling performance to fitness to ensure that ecologically-relevant parameters are being measured.

Studies measuring performance as a proxy for fitness should also carefully consider how they treat non-performing individuals. Many previous studies of performance in hatchling turtles either excluded non-performers from analysis, or assigned non-performers the time at which tests were censored. Dealing with non-performers in this way loses inter-individual variation and could result in erroneous conclusions, particularly because in some cases non-performance is actually advantageous (e.g., Janzen 1995). I suggest that future studies on performance use rank-based tests of individuals' performance, rather than direct measurements of performance times, in order to include non-performers in analyses.

In conclusion, I found that variation in daily temperature during incubation was positively correlated with speed of hatchling performance. In addition, performance was generally slower with cooler home climate. Forecasts of future climate change scenarios include increased frequency of extreme events (Solomon et al. 2007); therefore, it is likely that temperature fluctuations during the incubation period will increase. Increased daily thermal fluctuation may result in faster hatchling turtles, particularly at higher latitudes.

However, whether faster righting, sprinting, and swimming rates translate into enhanced fitness, and how these performance measures differ geographically, are yet to be determined.

Acknowledgements

Funding for this study came from the Iowa Academy of Sciences, Society for Integrative and Comparative Biology, Society for the Study of Amphibians and Reptiles and the Dean Metter Memorial Fund, Sigma Xi, and Iowa State University's Department of Ecology, Evolution, and Organismal Biology. Thanks to J. Clapp and M. Fritz for collecting permits, and C. Lee for hospitality at Bosque del Apache NWR. R. Alverio-Newton, K. Christensen, R. Clayton, E. Holman, N. Howell, J. Kubik, T. Mitchell, J. Stuart, H. Streby, J. Strickland, and D. Warner helped with animal collection and maintenance of the experimental ponds. This manuscript was much improved by valuable comments from A. Bronikowski, P. Dixon, F. Janzen, C. Kelly, E. Takle, H. Streby, and the Janzen Herpetology Lab at Iowa State University. All research was conducted in accordance with Institutional Animal Care and Use Committee protocol #1-09-6677-J (Iowa State University).

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Table 1. Mean mass and carapace length of hatchling *Chrysemys picta bellii* produced by females from five populations that nested in a common-garden in Story Co., IA in 2009. Populations are listed in order of increasing latitude. All values are means ± standard deviation.

Population	Number of hatchlings	Mass (g)	Carapace length (mm)
NM	19	3.81 ± 0.48	24.98 ± 1.32
IL	80	3.88 ± 0.56	24.75 ± 1.68
NE	12	3.24 ± 0.33	23.29 ± 0.64
IA	65	4.33 ± 0.51	25.46 ± 1.36
WA	27	4.30 ± 0.34	25.91 ± 1.43

Table 2. Median performance time (s) for righting, sprinting, and swimming by hatchling *Chrysemys picta bellii* produced by females from 5 populations that nested in a common-garden in Story Co., IA in 2009. Performance tests were censored at 180 s; therefore, in populations with median performance times of 180 s, > 50% of hatchlings failed to perform in a particular test.

Popn.	*N*	Latency to right	Active righting time	Total righting time	Latency to sprint	Active sprint time	Total sprint time	Latency to swim	Active swim time	Total swim time
NM	30	131.4	43.0	122.5	149.1	99.9	139.8	113.8	52.5	109.2
IL	128	151.6	65.2	151.6	155.9	123.3	158.6	156.6	74.9	161.4
NE	10	150.7	69.2	144.4	98.2	70.8	94.0	115.8	70.5	137.2
IA	122	169.4	78.0	170.1	155.5	115.3	154.3	161.0	75.2	154.8
WA	13	164.1	98.0	173.9	180.0	178.6	180.0	180.0	108.8	180.0

Figure legends

Figure 1. Median total performance time of hatchling painted turtles produced by females from five populations nesting in a common-garden environment in Story County, IA in 2009. The X-axis displays the 30-year mean May air temperature from each population's site of origin, with the corresponding populations indicated below the data points. Mean May air temperature generally decreased with latitude, except that the NE site has a cooler climate than the IA site despite being farther south. Populations differed in median sprint and swim times but not righting time, and both median sprint and swim times increased with latitude. Performance tests were censored at 180 s; therefore, in populations with median performance times of 180 s, > 50% of hatchlings failed to perform. Climate data are from the National Climate Data Center (www.ncdc.noaa.gov).

Figure 2. Clutch medians for total righting (A), sprinting (B), and swimming (C) time of hatchling painted turtles produced by females from five populations nesting in a common-garden environment in Story County, IA in 2009. Performance times decreased with mean daily thermal variance during the thermosensitive period of incubation; differences were significant for righting ($t_1 = 3.46$, $P < 0.01$) and swimming ($t_1 = 3.84$, $P < 0.01$; tobit regression with all performance tests censored at 180 s).

Figure 3. Nests with higher mean temperatures during the thermosensitive period of incubation produced hatchlings with a shorter latency to swim ($t_1 = -2.88$, $P = 0.036$; tobit regression censored at 180 s) in nests produced by females from five populations in a common-garden environment in Story County, IA in 2009.

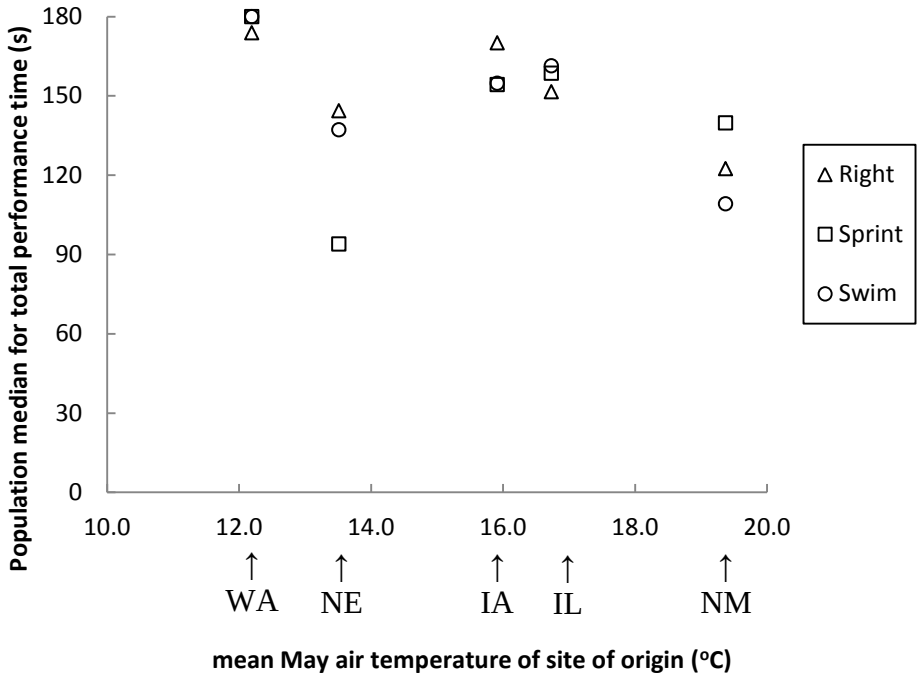
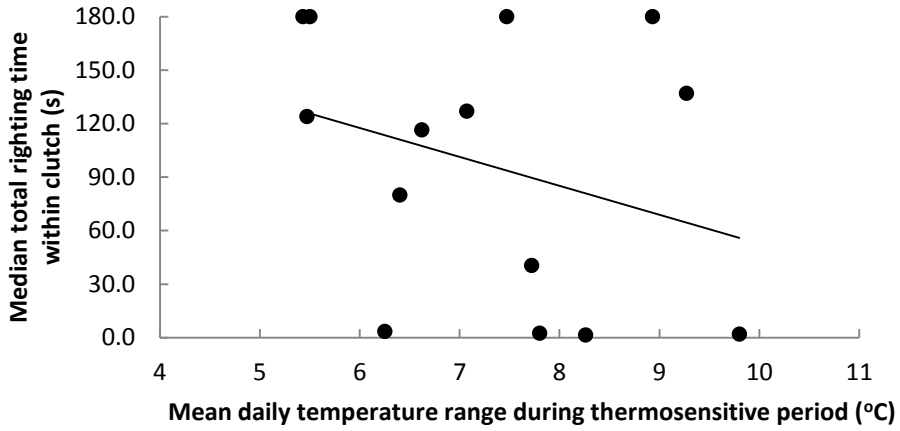
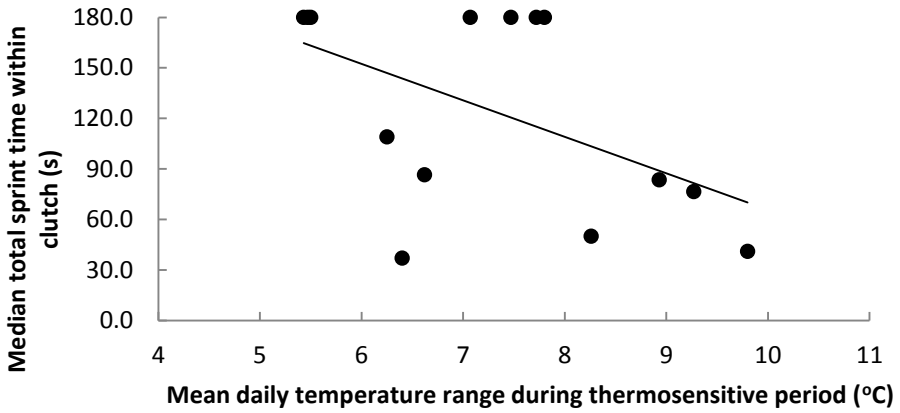

Population median for total performance time (s)
180
150
120
90
60
30
0
10.0
12.0
14.0
16.0
18.0
20.0
WA
NE
IA
IL
NM
mean May air temperature of site of origin (°C)
Right
Sprint
Swim

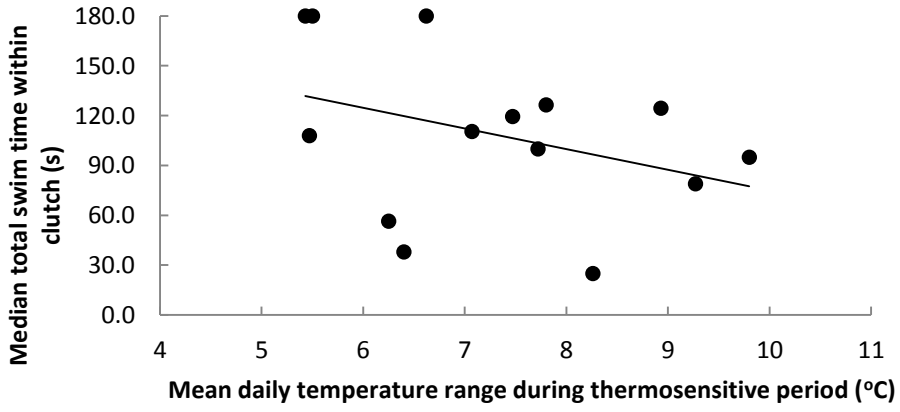
Figure 1


Median total righting time within clutch (s)
180.0
150.0
120.0
90.0
60.0
30.0
0.0
4
5
6
7
8
9
10
11
Mean daily temperature range during thermosensitive period (°C)

A


Median total sprint time within clutch (s)
180.0
150.0
120.0
90.0
60.0
30.0
0.0
4
5
6
7
8
9
10
11
Mean daily temperature range during thermosensitive period (°C)

B


Median total swim time within clutch (s)
180.0
150.0
120.0
90.0
60.0
30.0
0.0
4
5
6
7
8
9
10
11
Mean daily temperature range during thermosensitive period (°C)

C

Figure 2

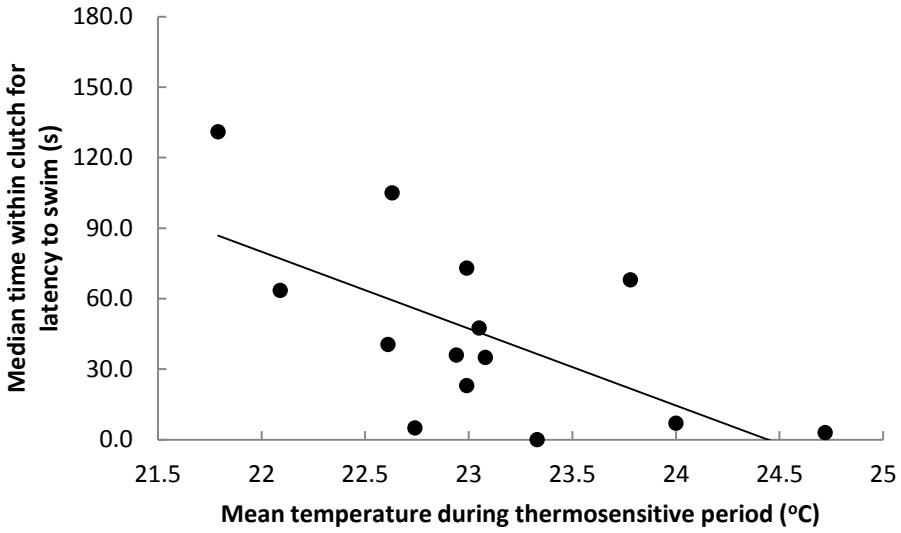

180.0
150.0
120.0
90.0
60.0
30.0
0.0
Median time within clutch for latency to swim (s)
21.5
22
22.5
23
23.5
24
24.5
25
Mean temperature during thermosensitive period (°C)

Figure 3

CHAPTER 5. ENVIRONMENTAL CONSTRAINTS ON NEST-SITE CHOICE: ARE TURTLE POPULATIONS LIMITED BY AVAILABILITY OF NESTING HABITAT?

A paper submitted for publication to *Global Change Biology*

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Abstract

Shifts in resource use may be an important mechanism by which organisms can adjust to novel environmental conditions, such as those imposed by climate change. However, for such shifts to be possible, environmental space must exist into which the organism can move. Habitat that ensures successful survival and reproduction is one such critical resource. We studied resource selection of shade cover over nest sites by painted turtles in populations in Illinois (center of range) and New Mexico (southern edge of range). We targeted this habitat feature because shade can influence hatching success and offspring phenotype (including sex in the study species) by affecting nest microenvironments. We found that while turtles in both populations selected nest sites that were shadier than average available sites, overall resource selection differed between the populations. This disparity was likely due to differences in vegetation structure that provides shade at each site, because areas with high shade cover in New Mexico (low dense thickets) were much more difficult for turtles to access than those in Illinois (dense tree canopy cover). Further, shade cover predicted different parameters of incubation regime at each site, suggesting that turtles must assess dissimilar components of shade cover in order to choose nest sites and predict their future incubation regimes. Our results suggest that shade cover within nesting areas is a key component of painted turtle habitat, and that accessible, highly-shaded nest sites are limited

at the New Mexico site. Maintaining a range of shade cover from which turtles can select nest sites would permit plasticity in nest-site choice to be expressed, which may be important in preventing sex ratio skews due to climate change.

Introduction

Under contemporary global climate change, organisms must adapt, adjust, or eventually perish (reviewed in Parmesan 2006; Sinervo *et al*. 2010). Adaptive changes in allele frequencies in response to novel climatic conditions have been demonstrated in some short-lived species (e.g., Bradshaw & Holzapfel 2001; Umina *et al*. 2005; Balanyá *et al*. 2006; Pen *et al*. 2010). More common, however, and particularly for longer-lived species, are shifts in phenology (e.g., Crick *et al*. 1997; Nussey *et al*. 2005; Pike *et al*. 2006; Todd *et al*. 2011) or geographic range (Parmesan *et al*. 1999, 2006; Chen *et al*. 2011). Such shifts may originate as phenotypic plasticity within or among individuals (Berteaux *et al*. 2004; Visser 2008), and may eventually become canalized over many generations (West-Eberhard 1989).

Regardless of its nature, in order for an ecological shift to alleviate an adverse biological effect of climate change, an environmental space must exist into which the organism can move (e.g., Walther *et al*. 2002). For example, if climatic warming limits a phytophagous insect's primary host plant, the insect could shift its host use to another species only if one is available. Similarly, corals and their zooxanthellae symbionts could colonize deeper waters as shallower habitats become too warm. However, beyond a certain depth, light intensity becomes limiting, and a shift to greater depths can no longer compensate for the effects of climate change on water temperature (Hoegh-Guldberg 1999). Understanding

the capacity of organisms to adjust is critical for predicting the potential effects of climate change on various taxa, but for conservation efforts to be effective, we also need to understand environmental constraints on such adjustments.

Species with temperature-dependent sex determination (TSD), in which the sex of offspring is irreversibly determined by the temperature at which embryos develop, may be particularly vulnerable to climate change for two main reasons. First, the sex-determining pathway is extraordinarily sensitive to environmental temperature, with ranges of only 1-2^{o}C separating the production of 100% male vs. 100% female offspring (Ewert *et al*. 1994). Second, organisms with TSD (e.g., crocodilians, tuatara, turtles, and some lizards and fish) tend to be relatively long-lived and slow to reproduce. Together, these characteristics of TSD species suggest that, while even minor changes in climatic temperatures could dramatically alter the sex ratio produced by a given population, such populations are unlikely to evolve quickly enough to keep pace with rapid climate change and thereby prevent sex ratio skews (Janzen 1994*a*, Morjan 2003*a*, McGaugh *et al*. 2010; but see McGaugh & Janzen 2011).

Behavioral plasticity in nest-site choice may prevent sex-ratio skews in some reptiles with TSD. For example, females may adjust the timing of nesting (Doody *et al*. 2006) or the depth of the nest chamber (Doody 2009; Telemeco *et al*. 2009) depending on environmental conditions. Yet in other TSD reptiles, adjusting the timing of nesting may be insufficient to compensate for effects of climate change on offspring sex ratio (Schwanz & Janzen 2008), and digging deeper nests is not feasible due to ecological constraints such as unavailability of sufficiently soft soil (Mitchell *et al*. 2008) or morphological constraints on female body size (Tiwari & Bjorndal 2000). In painted turtles, preference of shade cover over the nest site

may respond to selection in a climate-warming scenario (McGaugh *et al*. 2010), and choice of shade cover over the nest also appears to be behaviorally plastic in response to sudden, novel environmental conditions (Refsnider & Janzen in press). However, if a shift in preference for shade cover is a mechanism by which turtles may prevent sex ratio skews caused by a warming climate, a range in shade cover must be available in order for this plasticity to be expressed.

To determine whether choice of shade cover over a nest is ecologically constrained by availability of such habitat, we quantified the range of shade cover available in nesting areas used by populations of painted turtles in New Mexico, at the southern edge of the species' range, and Illinois, in the central portion of the species' range. The objectives of this study were to: 1) characterize the selection (i.e., use vs. availability) of shade cover at each site; 2) compare the use and availability of shade cover between sites; 3) determine whether limited shade cover availability acts as an ecological constraint on nest-site choice at either site, and 4) determine the relationship between shade cover and incubation regime at each site. We used the painted turtle as a model system to provide general insights into the constraining role that habitat availability could play in biological responses to climate change.

Materials and methods

Study species and site

We conducted this study using the western painted turtle, *Chrysemys picta bellii*. Painted turtles inhabit wetlands throughout the United States and southern Canada, and females emerge in May and June to nest in open areas such as beaches and lawns. Females

in some populations nest multiple times per season, whereas females in other populations nest only once per year. This species has Type 1a TSD, with females produced at constant incubation temperatures above 29^{o}C and males produced at constant temperatures below 27^{o}C (Ewert *et al*. 1994). We collected data in 2011 at the Thomson Causeway Recreation Area, Carroll Co., Illinois and Bosque del Apache National Wildlife Refuge, Socorro Co., New Mexico in known painted turtle nesting areas (e.g., Janzen 1994*b*; Morjan 2003*b*).

Availability of shade cover

We quantified shade cover availability at each site before the onset of nesting (17-18 May and 25-30 May at the Illinois and New Mexico sites, respectively). At each site, we established transects through the known nesting area. Transects were 5 m apart and began at the edge of a wetland. Every 5 m along each transect, we took a hemispherical photograph using a 180^{o} fisheye lens. We then quantified the per cent shade cover over each point using Gap Light Analyzer (Frazer *et al*. 1999). Topographic differences caused the shape of the sampled area to differ between sites (i.e., roughly triangular for the Illinois site and rectangular for the New Mexico site), but the overall area sampled was similar at both sites (9921 m^2 and 9704 m^2 for Illinois and New Mexico respectively; Fig. 1).

Incubation regime

We installed data loggers (iButton, Embedded Data Systems, Lawrenceburg, KY) at some sampling points at each site to measure incubation regimes at potential nest sites. We buried loggers at the population mean nest depth for the appropriate site (8.7 and 10.7 cm for the Illinois [Chapter 7] and New Mexico sites [Morjan 2003*b*], respectively) and recorded temperature hourly for 90 days. We installed 24 loggers at randomly-assigned sampling points at the Illinois site. At the New Mexico site, due to extensive dredging activities

around the canals running through the nesting area, we were unable to install loggers randomly throughout the sampled area. Instead, we used a subsampling design at this site and installed 24 loggers in a 5 x 5 m grid within the larger sampled area (Fig. 1).

We retrieved loggers and downloaded temperature data in September 2011. To quantify the incubation regime at each point containing a data logger, we calculated the following four incubation regime parameters: minimum temperature, maximum temperature, mean daily temperature, and mean daily range of temperature (i.e., mean of the highest – lowest temperature recorded during each 24-hour period; Ashmore & Janzen 2003; Morjan & Janzen 2003). These four parameters were determined separately for June and July for each sampling point.

Use of shade cover

We observed painted turtle nesting behavior by patrolling nesting areas hourly from 23 May - 30 June at the Illinois site and 23 May – 29 June at the New Mexico site. We monitored nesting turtles from a distance to prevent nest abandonment due to disturbance. Upon completion of nesting, we took a hemispherical photograph with the camera placed directly on top of the nest cavity, and analyzed per cent shade cover over the nest as described above.

Statistical analyses

We conducted all statistical analyses using SAS 9.2 (SAS Institute, Cary, North Carolina). At each site, the distributions of used and available shade cover were approximately normal. Within each site, we compared the means of the distribution of shade cover over used and available sites using a *t*-test. Similarly, we compared the means of the

distribution of shade cover at used sites and at available sites between Illinois and New Mexico using a *t*-test.

To compare turtles' selection of shade cover in the two study populations, we defined the resource selection function (as in Manly *et al*. 1993) for each population as:

$\omega(x) = \exp(\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \ldots)$,

where $\omega(x)$ is the relative probability that a point with covariate vector x is used as a nest site. We estimated the coefficients (β_1, β_2, $\beta_3 \ldots$) using standard logistic regression, with used sites coded as 0 and available sites coded as 1, and percent shade cover as the independent variable. To identify the set of covariates that best described selection of shade cover in each population from the distribution of available shade cover, we considered models including shade cover, the square of shade cover (to determine whether the relationship between shade cover and selection probability was non-linear), and both shade and shade squared (as in Erickson *et al*. 1998); we chose the model with the lowest AIC_c value as the best model. For each population, we calculated the resource selection function at each point at which shade cover was quantified. We interpolated the resource selection function across the sampled area at each site using the spline function in the 3D Spatial Analyst in ArcMap (ESRI, Redlands, CA).

We used linear regression to assess how well shade cover predicted 4 parameters of incubation regime at each study site. For each study site, we conducted 8 separate regressions with shade cover as a predictor and 4 parameters of incubation regime, in both June and July, as response variables. All *P*-values for linear regressions were corrected for multiple testing ($m = 8$) using the Bonferroni adjustment. All values are shown as means ± standard deviation.

Results

At both the Illinois and New Mexico study sites, selected nest sites had more shade cover than the average available site (IL: $t_{293,214} = -7.18$, $P < 0.0001$; NM: $t_{300,8} = -2.52$, $P = 0.041$; Table 1). On average, the Illinois site had greater availability of shade cover than the New Mexico site ($t_{300,292} = 16.79$, $P < 0.0001$), and turtles in Illinois selected nest sites with greater shade cover than nest sites selected by turtles in New Mexico ($t_{214,9} = 6.01$, $P = 0.0002$).

The logistic regression model that best described selection of nest sites from the distribution of available shade cover in Illinois included only shade cover as a covariate. The resource selection function for Illinois, shown in Figure 2a, was

$\omega(x) = e^{(0.054 * \text{shade})}$.

For the New Mexico population, both shade cover and shade cover squared were included in the best logistic regression model. The resource selection function for New Mexico, shown in Figure 2b, was

$\omega(x) = e^{(0.4180*\text{shade}) + (-0.0062*\text{shade}\wedge 2)}$.

Overall, the relative probability of selection increased with increasing shade cover at the Illinois site. In contrast, in New Mexico, relative selection probability increased until reaching a maximum around 40% shade cover, and then decreased as shade cover further increased (Fig. 2). Probability of selection relative to shade cover availability throughout the sampled area at each study site is shown in Figure 3.

Shade cover predicted somewhat different parameters of the incubation regime of available sites in Illinois compared to New Mexico. In Illinois, shade cover was negatively correlated with minimum temperature in June ($F_{1,22} = 12.44$, $P = 0.015$, $R^2 = 0.36$) and July

($F_{1,22}$ = 21.03, P = 0.008, R^2 = 0.49). No other relationships between shade cover and incubation regime at the Illinois site were statistically significant (all P's > 0.70). In contrast, in New Mexico, shade cover was negatively correlated with mean incubation temperature in June ($F_{1,22}$ = 13.95, P = 0.009, R^2 = 0.39) and July ($F_{1,22}$ = 20.23, P = 0.002, R^2 = 0.48), mean daily temperature range in July ($F_{1,22}$ = 10.77, P = 0.027, R^2 = 0.33), and maximum temperature in June ($F_{1,22}$ = 9.86, P = 0.038, R^2 = 0.31) and July ($F_{1,22}$ = 15.53, P = 0.006, R^2 = 0.41; Fig. 4). All P-values reflect Bonferroni adjustments.

Discussion

Ecological shifts may be important mechanisms in allowing organisms to adjust to novel environmental conditions caused by climate change. However, for an ecological shift to allow an organism to adjust to some aspect of climate change, an environmental space must exist into which the organism can shift. In painted turtles, choice of shade cover over nest sites is a behaviorally plastic characteristic (Refsnider & Janzen in press) that can affect offspring sex (Janzen 1994*b*), post-hatching growth (Janzen & Morjan 2002), and other important traits (Warner *et al*. 2010) by influencing the microenvironments within nests (Weisrock & Janzen 1999; Morjan & Janzen 2003; Schwanz *et al*. 2010). In order for this plasticity to be expressed, a range of shade cover options must be available from which females can select a nest site. Our results show that selection of nest sites from available habitat differs between populations. Moreover, the relationship between shade cover and incubation regime at potential nest sites differed substantially between the two study sites.

Importantly, turtles in both populations selected nest sites that were shadier than the average available site, supporting shade cover as a key component of painted turtle nesting

habitat. In species with TSD, nest microhabitat affects not only overall survival of offspring, but many measures of offspring phenotype, including sex (reviewed in Refsnider & Janzen 2010). Our results demonstrate that turtles select nest sites non-randomly with respect to shade cover, and that shade cover reliably predicts at least some components of the site's future incubation regime. Because choice of shade cover over nest sites is plastically adjustable in response to changing environmental conditions (Refsnider & Janzen in press), the continued availability of shaded nest sites is likely to become increasingly important as ambient temperatures rise. An adequate range of shade cover from which females can select nest sites will be important not only in mitigating sex-ratio skews in species with TSD, but also in preventing developmental abnormalities and recruitment failure (Janzen 1994*a*) due to extreme nest microenvironments.

Although turtles in both populations selected nest sites that were shadier than the average available site, the resource selection function for shade cover differed between the study sites. At the Illinois site, relative probability of use continued to increase with increasing shade cover. However, as the greatest shade cover measured in the Illinois nesting area was 77%, extrapolation of the resource selection function beyond the observed data may be inaccurate. Therefore, selection for shade cover may not continue to increase with greater than 80% shade cover. In contrast, in New Mexico, the relative probability of use increased up to about 40% shade cover, and then declined with further shade cover. This difference in resource selection between sites is likely because the structure of the vegetation producing shade was different at the two sites. In New Mexico, shade cover was provided by scattered shrubs and dense thickets of willow (*Salix* sp.). Sites with the greatest shade cover were usually those in willow thickets, which may not be suitable for nesting because the density

and rigidity of vegetation at ground level is likely difficult for female turtles to travel through or dig in. Indeed, previous research on the New Mexico population also showed that turtles did not nest in the most highly shaded areas (Morjan 2003*b*). In contrast, in Illinois, sites with greater shade cover were open at ground level but shaded most of the day by the foliage of tall trees. This difference in vegetation structure suggests that, although the overall amount of shade present may not serve as a constraint in either population, shade actually available to nesting turtles is likely a limiting resource at the New Mexico site (Fig. 5). Previous research at the New Mexico site found that turtles nested at sites with less south and west vegetation cover than random sites (Morjan 2003*b*). Rather than quantifying shade cover at random sites, our study censused shade cover available throughout a 9704 m^2 area encompassing nesting habitat. By sampling intensively over a large area, our study design allowed us to detect differences between used and unused but available sites at a larger scale than was previously possible.

That the two study populations differ in selection of shade from available habitat due to differences in vegetation structure is supported by the between-site differences in the relationship between shade cover and incubation regime. Shade cover over randomly-assigned, potential nest sites predicted minimum soil temperature at the Illinois site, while in New Mexico shade cover predicted maximum temperature, mean temperature, and mean daily range of temperature. These different relationships between shade cover and potential incubation regime suggest that selection of nest sites from available habitat has site-specific consequences. For example, in New Mexico, an increase in shade cover results in a sharp decrease in mean daily temperature, mean daily temperature range, and maximum temperature in July. In Illinois, however, the same increase in shade cover has little or no

relationship with those measures of incubation regime. Therefore, the selection of a nest site from a range of available shade cover in New Mexico ensures that the shade cover at the time of nest-site choice strongly predicts incubation regime. In Illinois, however, this relationship is much weaker, and may require turtles to spend more time assessing potential nest sites for some characteristic that predicts nest incubation regime. Indeed, at the Illinois study site, solar irradiance from the south and west over selected nest sites predicted offspring sex ratio (Janzen 1994*b*; Schwanz *et al*. 2010). Turtles in Illinois may need to assess shade cover from particular directions to accurately predict incubation regime at potential nest sites, whereas in New Mexico overall shade cover appears to provide sufficient predictive power of future incubation regime. That turtles at the Illinois site are selecting nest sites with characteristics that predict future incubation regime is supported by previous studies at that site, which found that shade cover from the south and west predicts incubation regime at nest sites (e.g., Janzen 1994*b*; Weisrock & Janzen 1999; Morjan & Janzen 2003; Schwanz *et al*. 2010). Because results from our study suggest that shade cover does not reliably predict incubation regime at a variety of available but *unused* sites in Illinois, and previous studies at the same site have found that shade cover predicts incubation regime at *used* nest sites (e.g., Janzen 1994*b*; Morjan & Janzen 2003; Schwanz *et al*. 2010), it is likely that turtles at the Illinois site are selecting nest sites with characteristics that predict future incubation regime. Available but unused sites may lack such predictive characteristics, which would explain why shade cover is a better predictor of future incubation regime at sites selected for nesting than available sites in general.

Ecological shifts that allow organisms to adjust to changing environmental conditions could occur at large scales, such as a species that shifts its range poleward, or at much

smaller scales, such as individuals selecting microhabitats with different abiotic conditions. Poleward range shifts in response to climate change have been observed in a variety of taxa (e.g., Parmesan *et al*. 1999; Hickling *et al*. 2006). Shifts in microhabitat use are more difficult to quantify, but could be of critical importance to the persistence of organisms despite a changing climate. For example, in green turtles, offspring sex ratio is driven mainly by the color of sand on nesting beaches: light-colored beaches have a greater albedo effect than beaches with darker sand, and are therefore cooler. Because green turtles have Type 1a TSD like painted turtles, producing males at cooler incubation temperatures, lighter-colored beaches will become increasingly important as the climate warms because they are the most likely to begin producing female offspring (Hays *et al*. 2003); therefore, light-colored beaches could be a target for sea turtle conservation efforts. In other cases, reducing the availability of ecological space could prevent the spread of invasive species or pathogens as climate change creates otherwise more favorable environmental conditions for such species. For example, although the range of dengue-carrying mosquitoes in Australia is predicted to increase due to climate change, the magnitude of increase depends on availability of breeding habitat (Kearney *et al*. 2009), over which humans have some control.

Understanding how the availability of specific resources may limit species' ability to adjust to changing environmental conditions will inform adaptive management practices. For example, if breeding habitat for dengue-carrying mosquitoes in Australia is entirely restricted to small, rain-collecting containers rather than larger reservoirs, the extent of the mosquito's range expansion will be minimized (Kearney *et al*. 2009). Results of our study suggest that in New Mexico, habitat with high levels of shade that is accessible to nesting turtles (i.e., not located in dense thickets) may be limited. To increase the range of available, suitable nesting

habitat at the New Mexico site, managers could plant scattered native shrubs throughout the nesting area. Shade cover does not appear limited at the Illinois site, but it will be important to maintain the range of shade cover currently present at this site. Managers at this site should replace trees that die, and the occasional planting of additional trees as summer temperatures increase will likely ensure that adjustment of nest-site choice by turtles can keep pace with climate change. By maintaining a range of shade cover options in nesting areas used by turtles, plasticity for choice of shade cover over nests can be expressed, and thereby potentially allow turtles, a globally imperiled taxon (IUCN 2011), to minimize skews in offspring sex ratio and other deleterious effects caused by climate change. Our results suggest that key habitat features related to sex ratio vary geographically within species. Future research should focus on identifying these key habitat features among populations of other species, particularly for those populations that are most vulnerable to habitat alteration and climate change.

Acknowledgements

This study was funded by the William Clark Graduate Student Award in Ecology and Evolutionary Biology (to J.M.R.) and NSF grant DEB-064932 (to F.J.J.). Thanks to the U.S. Army Corps of Engineers for access to, and H. Streby for help with data collection at, the Illinois site, and to A. Inslee and the staff at Bosque del Apache National Wildlife Refuge for hospitality at the New Mexico site. A. Bronikowski, P. Dixon, C. Kelly, E. Takle, and H. Streby provided helpful comments on earlier drafts of the manuscript. This research was conducted in accordance with Institutional Animal Care and Use Committee protocols # 12-03-5570-J and 6-08-6583-J (Iowa State University), Scientific Collecting Permits 3430 (New

Mexico Department of Game and Fish) and NH10.0073 (Illinois Department of Natural Resources), and Special Use Permit 32576-OA022 (U.S. Fish and Wildlife Service).

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Table 1. Mean shade cover over available and used sites within nesting habitat of *C. picta bellii* at Thomson Causeway Recreation Area (Carroll County, IL) and Bosque del Apache National Wildlife Refuge (Socorro County, NM), measured in 2011.

	Mean (%)	**Range (%)**	**Coefficient of variation (%)**
Illinois			
Available (*N* = 293)	47.7 ± 13.2	15.4 – 77.1	27.7
Used (*N* = 214)	55.6 ± 10.7	29.98 – 77.5	19.3
New Mexico			
Available (*N* = 301)	22.4 ± 22.5	0 – 63.6	100
Used (*N* = 9)	32.5 ± 11.4	15.7 – 46.2	35.1

Figure legends

Figure 1. Study sites at Thomson Causeway Recreation Area (Carroll County, IL; a) and Bosque del Apache National Wildlife Refuge (Socorro County, NM; b) in 2011. For each site, nesting areas used by *C. picta bellii* are enclosed in outlined area. Areas within which shade cover was measured in a 5 x 5 m grid are outlined in bold. Dots are locations at which incubation temperature was measured using data loggers inserted in the soil. For scale, the nesting area at the IL site is 120 m across at the center, and the nesting area at the NM site is ~50 x 300 m.

Figure 2. Use, availability, and resource selection function of shade cover within nesting habitat of *C. picta bellii* at Thomson Causeway Recreation Area (Carroll County, IL; a) and Bosque del Apache National Wildlife Refuge (Socorro County, NM; b), measured in 2011.

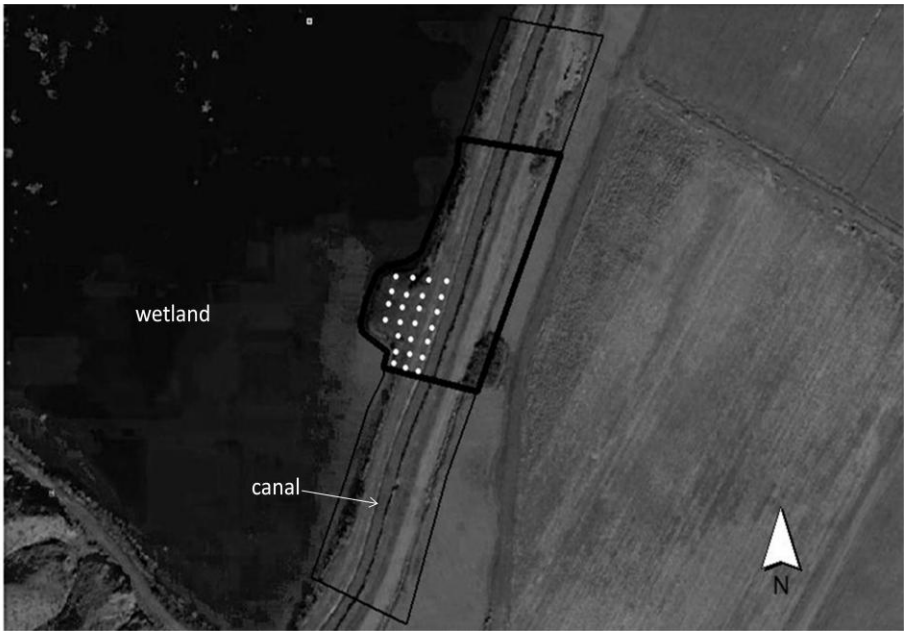
Figure 3. Probability of selection of shade cover relative to availability in painted turtle nesting habitat at Thomson Causeway Recreation Area (Carroll County, IL; a) and Bosque del Apache National Wildlife Refuge (Socorro County, NM; b, measured in 2011. Shade cover was quantified using temperature loggers and the resource selection function was calculated at the colored points shown, and the relative probability of selection was then interpolated throughout the sampling area using a spline function. Warm colors indicate low relative probability of selection, and cool colors indicate high relative probability of selection.

Figure 4. Relationship between % shade cover and 4 parameters of incubation regime (in ^{o}C) measured at 24 randomly-assigned points within nesting habitat of *C. picta bellii* at Thomson Causeway Recreation Area (Carroll County, IL; open circles and dotted lines) and at Bosque del Apache National Wildlife Refuge (Socorro County, NM; closed circles and

solid lines) in July 2011. Shade cover was negatively correlated with mean July temperature (a), mean daily range of temperature (b), and maximum July temperature (d) in New Mexico; and with minimum July temperature (c) in IL only.

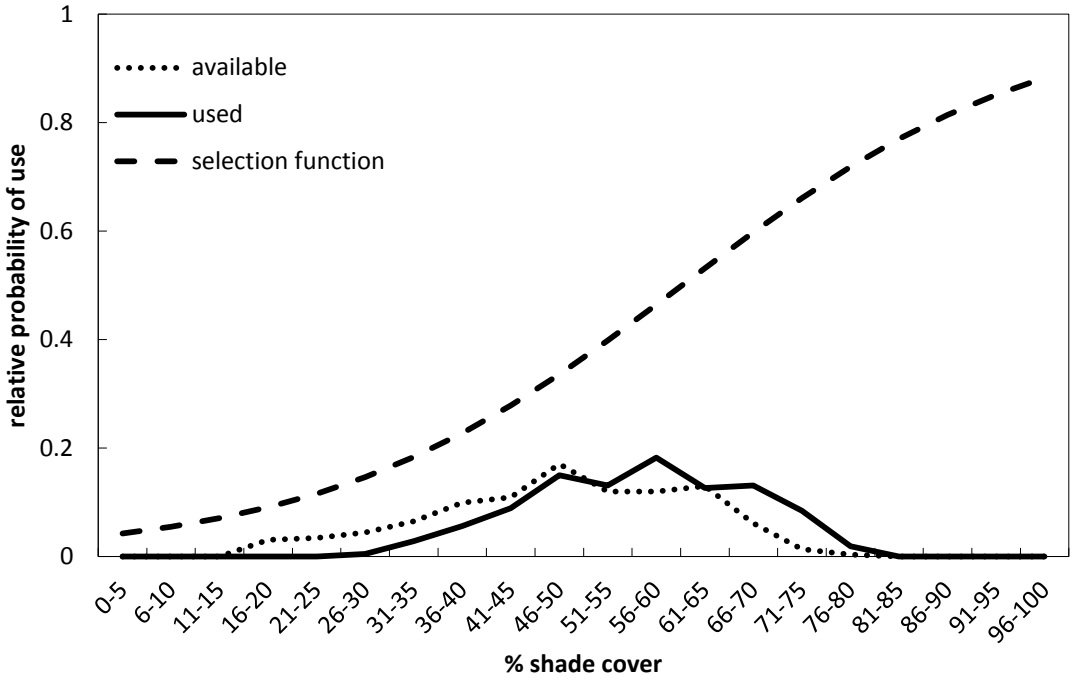
Figure 5. Shade structure in nesting habitat at Thomson Causeway Recreation Area (Carroll County, IL; a) and Bosque del Apache National Wildlife Refuge (Socorro County, NM; b), measured in 2011. Within shaded areas, note the openness at ground level at the Illinois site, compared to the dense vegetation at ground level (i.e., in the willow thicket) at the New Mexico site.

(a)



(b)

Figure 1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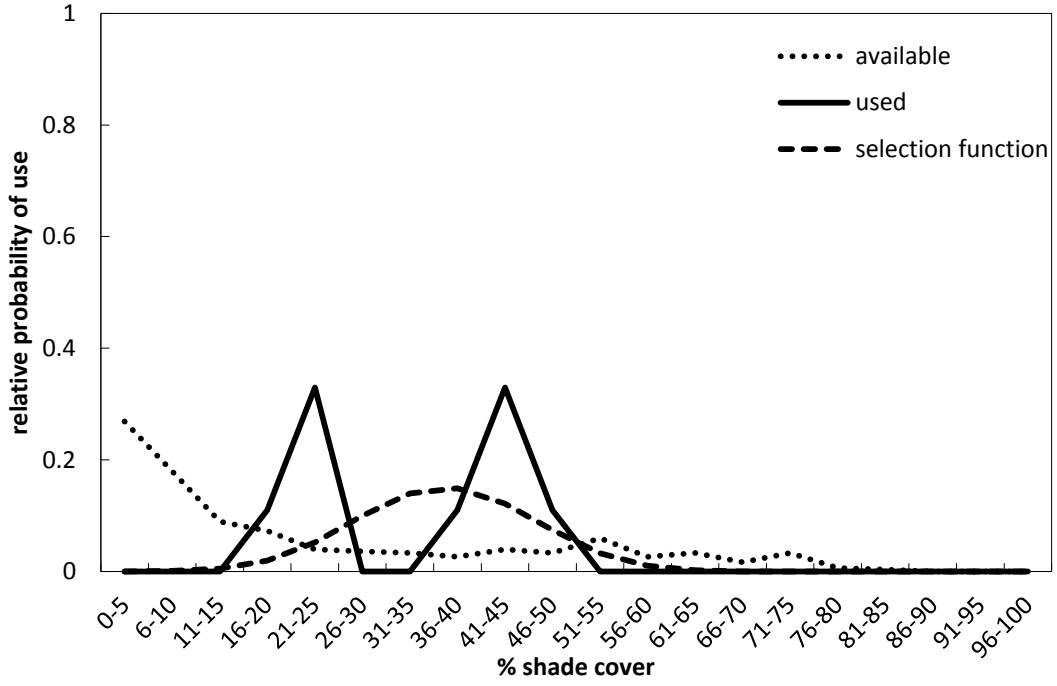
% shade cover

(a)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% shade cover

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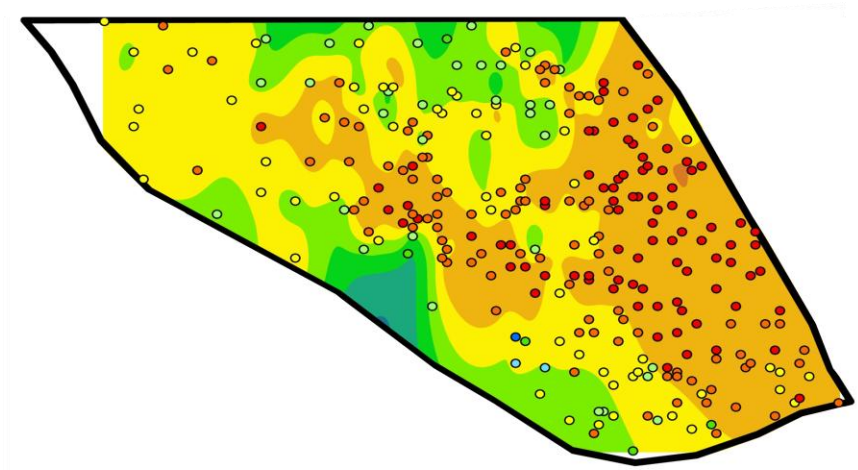
Figure 2



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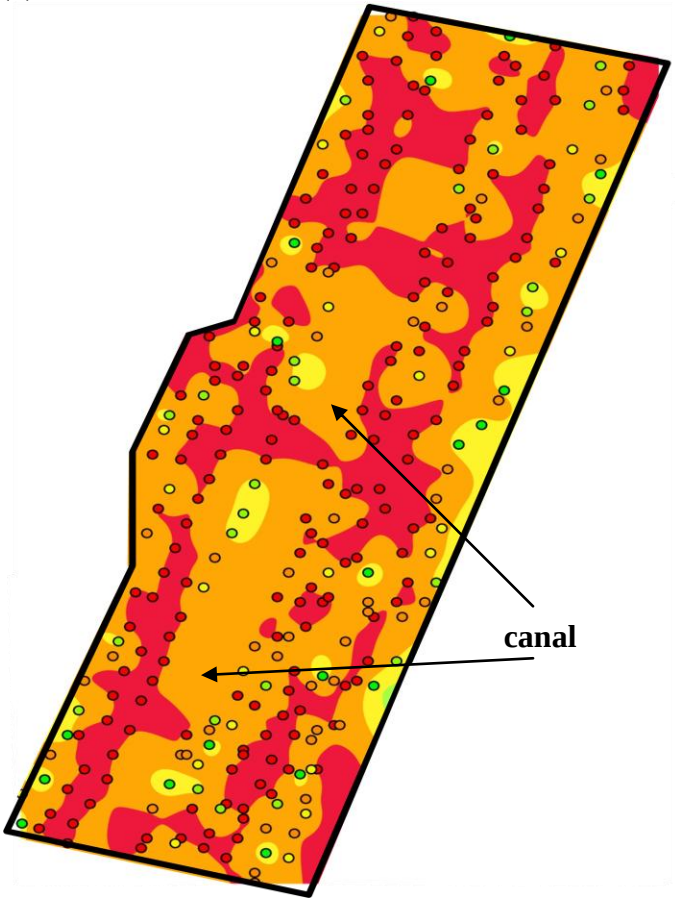

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Figure 3

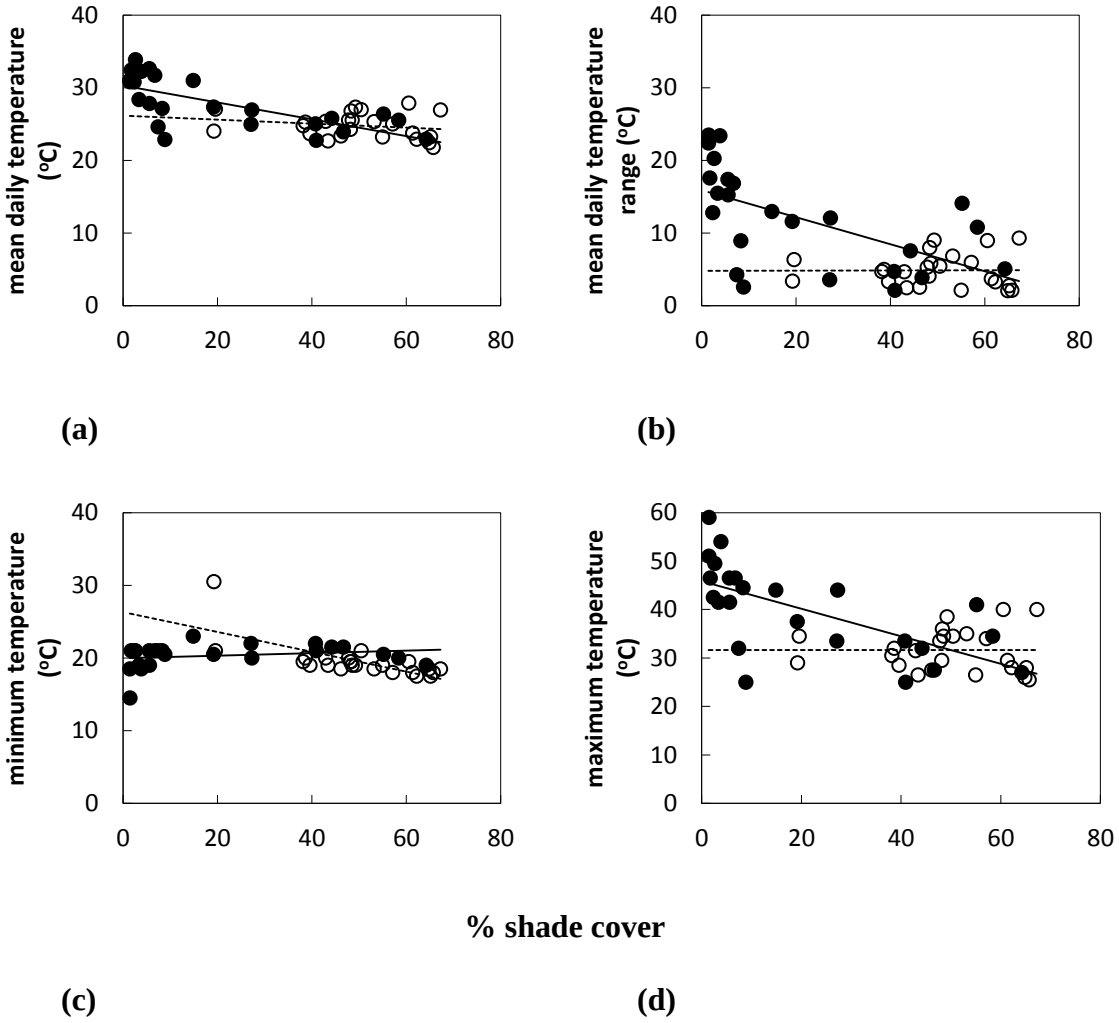
mean daily temperature (ºC)
mean daily temperature range (ºC)
minimum temperature (ºC)
maximum temperature (ºC)
(a)
(b)
(c)
(d)
% shade cover

Figure 4

(a)

(b)

Figure 5

CHAPTER 6. DOES FEMALE BODY SIZE CONSTRAIN NEST DEPTH IN FRESHWATER TURTLES?

A paper submitted for publication to *Journal of Herpetology*

Jeanine M. Refsnider

Abstract

Adjustment of nest depth has been proposed as a mechanism by which reptiles with temperature-dependent sex determination may be able to keep pace with climate change-induced increasing soil temperatures during egg incubation. However, morphological constraints such as female size may limit maximum nest depth, particularly in turtles. I used several measures of female body size and nest-site characteristics to predict nest depth in a southern and a central population of painted turtles, *Chrysemys picta*. I found that female body size, and, to a lesser extent, clutch size, are important predictors of nest depth. Nest depth in painted turtles appears to be constrained by female body size because smaller females have shorter rear limbs with which to excavate the nest cavity. Females from the southern site constructed deeper nests than females from the central site, and females from the southern site also appear to be constructing nests with depths closer to females' maximum physical capability compared to females from the central site. My results suggest that southern females may have less capacity to compensate for a warming climate through construction of deeper nests than females from the center of the species' range.

Introduction

Contemporary climate change is now recognized as affecting numerous life-history traits, including phenology (e.g., Beebee, 1995; Crick et al., 1997; Nussey et al., 2005; Pike et al., 2006), reproductive output (Reading, 2007; Tucker et al., 2008), disease resistance (Kutz et al., 2005), and ability to locate necessary resources (Ujvari et al., 2011). Reptile species are predicted to be particularly vulnerable to climate change because they are generally unable to shift their geographic distribution (Mitchell and Janzen, 2010). The difficulty of thermoregulating under increasing temperatures will likely lead to reduced activity and a decline in performance in many lizard species (e.g., Huey and Tewksbury, 2009; Kearney et al., 2009), and skews in sex ratios are predicted in species with temperature-dependent sex determination (TSD) such as turtles and tuatara (e.g., Janzen, 1994; Hawkes et al., 2007; Mitchell et al., 2008).

Behavioral plasticity in nest-site choice has been proposed as a mechanism by which reptiles with TSD may prevent sex ratio skews despite climatic warming (Bulmer and Bull, 1982). For example, females may adjust where they nest (Doody et al., 2006; Refsnider and Janzen, in press), the timing of nesting (Zani, 2008; but see Schwanz and Janzen, 2008), or the depth of the nest cavity (Doody, 2009; Telemeco et al., 2009) to compensate for environmental conditions. Indeed, in some species nest depth affects incubation conditions (Roosenburg, 1996; Leslie and Spotila, 2001) and therefore may influence sex ratio. However, the capacity to adjust nest depth likely varies among reptile taxa. Lizards and tuatara dig nests by burrowing downward with their front limbs; thus, the depths of their nests are likely constrained by substrate characteristics (Mitchell et al., 2008). In contrast, turtles construct nests using their rear limbs, and consequently nest depth in turtles is likely

constrained by rear limb length (Doody and Moore, 2010). Therefore, adjustment of nest depth by turtles in response to climatic warming may be less likely than in reptiles that construct nests using their front limbs.

Rear limb length is thought to constrain nest depth and thus clutch size in sea turtles (Tiwari and Bjorndal, 2000) and, in a common-garden experiment on nest-site choice in painted turtles, nest depth differed among populations due to female body size (Refsnider and Janzen, in press). These studies suggest that nest depth is a non-plastic character that, in order to shift, would require a concurrent evolutionary shift in female body size. However, nest depth in turtles may be somewhat adjustable in response to environmental conditions: in the painted turtle, mean depth of nests in a southern population is ~2 cm deeper than nests in a central population (Morjan, 2003), suggesting that the southern population compensates for a warmer local climate by constructing deeper nests. To assess the potential of turtles to keep pace with climate change by increasing nest depth, I sought to determine whether nest depth is constrained by female body size in the painted turtle. I compared female body size and nest depth in a southern and a central population. I then used several parameters associated with female size and nest-site choice in a model to predict nest depth and to disentangle the effects of female size and clutch size on nest depth. Finally, I assessed how close to the maximum possible nest depth females from each population are currently constructing nests as a proxy for their capacity to compensate for effects of climate change via shifts in nest depth.

Materials and Methods

Study site and species.— This study was conducted using the western painted turtle, *Chrysemys picta bellii*. Painted turtles inhabit a wide variety of aquatic habitats across the United States and southern Canada; the western subspecies occurs primarily west of the Mississippi River. Female turtles emerge from wetland habitats in May and June to nest in open areas (commonly beaches and lawns). During nest construction, female painted turtles prop themselves up with their front limbs, and use their back limbs to dig and shape the nest cavity, arrange eggs within the nest, and finally to refill the top of the nest with soil (Fig. 1).

Data Collection.— I collected data on female size and nest-site choice at southern and central study sites within the western painted turtle's geographic range. The southern site was Bosque del Apache National Wildlife Refuge, Socorro Co., New Mexico, and the central site was Thomson Causeway Recreation Area, Carroll Co., Illinois. I monitored known nesting areas during evening hours in May and June of 2010 (both sites) and 2011 (New Mexico site only), observing nesting females from a distance to prevent disturbance. Following completion of nesting, I captured females briefly to measure carapace and plastron length and width. For a subset of females at each site, I also measured the length of both rear limbs as the length of the fully-extended rear limb from the margin of the plastron to the tip of the longest claw; the mean of both rear limbs was used in statistical analyses. I then carefully excavated the nest to determine clutch size and to measure total nest depth. Finally, I took a hemispherical photograph over each nest site and quantified shade cover over nests using Gap Light Analysis software (Frazer et al., 1999).

Statistical Analyses.— While rear limb length is likely the morphological characteristic that most directly affects nest depth, it is a difficult character to measure and

variability likely exists among measurements taken by different researchers. I regressed mean rear limb length against plastron length for 83 females from the Illinois population nesting in 2010 and for 10 females from the New Mexico population nesting in 2010 and 2011. Mean rear limb length was positively correlated with plastron length ($F_{1,91} = 10.92$; $P < 0.01$; Fig. 2); therefore, in further analyses, I use plastron length as a measure of female body size.

I conducted all statistical analyses using SAS 9.2 (SAS Institute, Cary, North Carolina). I analyzed among-population differences in female body size (i.e., plastron length), clutch size, shade cover over the nest, and nest depth using analysis of variance. To predict nest depth, I used general linear mixed-model analysis of variance in the MIXED Procedure, using maximum likelihood estimation, with population, female size, clutch size, and female size x clutch size interaction as independent predictors, and shade cover as a random effect. Candidate models were ranked using Akaike's Information Criterion (AIC; Burnham and Anderson, 2002). I considered the best-supported model and all models with $\Delta AIC < 2.0$ to be competing models. To disentangle the effects of female size and clutch size on nest depth, I tested for between-population differences in the slopes of nest depth regressed on female size and clutch size using Type III sum-of-squares in analysis of covariance in the GLM Procedure. Finally, for each population separately, I used the maximum observed values for female body size and clutch size to predict the maximum expected nest depth; I qualitatively compared these values to the mean observed nest depth to assess how close to their capacity female turtles in each population are currently constructing nests. All values are presented as means ± standard deviation.

RESULTS

I collected data on female size and associated nest depths for 10 females from the southern population (NM) and 290 females from the central population (IL). The two populations did not differ in mean female plastron length (NM: 154.2 ± 11.0 mm, IL: 153.7 ± 11.7 mm; $F_{1,289} = 0.01$; $P = 0.93$) or clutch size (NM: 9.1 ± 2.1, IL: 10.1 ± 2.3; $F_{1,297} = 1.46$; $P = 0.23$). However, nests in the southern population were significantly deeper (NM: 92.5 ± 13.5 mm, IL: 83.5 ± 12.6 mm; $F_{1,299} = 5.44$; $P = 0.02$) and constructed under significantly less shade cover (NM: 28.1 ± 14.2%, IL: 55.1 ± 10.8%; $F_{1,298} = 63.3$; $P < 0.0001$) than nests in the central population.

The best evaluated models of nest depth contained the predictors Population, Female size, Clutch size, and Female size * clutch size interactions (all within $\Delta AIC < 2.0$). As female size and clutch size strongly co-vary ($r = 0.44$; $F_{1,287} = 68.89$; $P < 0.0001$), I compared the slopes of nest depth regressed on both female size and clutch size to disentangle the effects of these two variables on nest depth. Although the slope of nest depth regressed on female size did not differ from that of nest depth regressed on clutch size ($P = 0.42$; Fig. 3), the correlation coefficient for the relationship between nest depth and female size ($r = 0.37$) was greater than that for the relationship between nest depth and clutch size ($r = 0.28$), indicating a stronger correlation between nest depth and female size.

As a rough estimate of each population's maximum possible nest depth, I used the multiple regression line of best fit including coefficients for female size (in mm) and clutch size (number of eggs) for each population separately. The equation for the New Mexico population was

nest depth = (-0.2491*female size) + (5.3414*clutch size) + 79.5305

and that for the Illinois population was

nest depth = (0.3135*female size) + (0.8415*clutch size) + 26.6463.

Then, using the maximum observed values for female size and clutch size in each population, I estimated the maximum possible nest depth for each population. The maximum observed values for female size and clutch size in the New Mexico population were 175 mm and 12 eggs; those for the Illinois population were 184 mm and 18 eggs. The resulting estimates for maximum possible nest depth were 100 mm for the New Mexico population and 99 mm for the Illinois population. Comparing each population's current mean nest depth with the calculated maximum possible nest depth, the Illinois population is constructing nests with depths at 84% of their capacity, while the New Mexico population is constructing nests with depths at 92% of their capacity.

DISCUSSION

Adjusting nest depth may provide a mechanism by which female turtles could influence the incubation regime experienced by developing embryos. In theory, females may be able to compensate for unusually warm environmental temperatures by constructing deeper, cooler nests; however, if nest depth is constrained by body size, females may be unable to increase nest depth without evolving a larger body size. This study reveals that nest depth in painted turtles is strongly influenced by female body size. Moreover, the depths at which females construct nests are currently near their physical capacity, particularly in a southern population.

The unique morphology of the shell imposes constraints on certain reproductive characteristics in turtles. For example, width of the pelvic aperture constrains egg size in

some smaller freshwater turtles (Congdon and Gibbons, 1987; Clark et al., 2001). Similarly, Tiwari and Bjorndal (2000) suggest that rear limb length in sea turtles indirectly constrains clutch size by limiting nest depth and thereby the space within the nest cavity available for eggs. My results support the idea that nest depth is constrained by female body size in turtles because the length of the rear limbs limits the depth to which a female can dig a nest cavity. Clutch size was also an important predictor of nest depth, which is not surprising as clutch size strongly co-varies with female body size, but because the relationship between female size and nest depth was stronger than that of clutch size and nest depth, female size appears to exert the greatest constraint on nest depth. An experiment that artificially manipulates clutch size (sensu Du et al., 2005) would be necessary to establish the causal relationships between body size, clutch size, and nest depth in turtles.

In both this study and that of Morjan (2003), turtles in New Mexico constructed significantly deeper nests than turtles in Illinois. Deeper nests in New Mexico may compensate for warmer air temperatures at that site compared to Illinois, particularly as females in New Mexico nested under less shade cover than females in Illinois (Refsnider et al., unpubl. data). New Mexico females also appear to construct nests closer to their estimated maximum capacity than do Illinois females, which again suggests that females in New Mexico may be digging deeper nests to compensate for a warmer climate. If this is the case, southern females may have less ability to adjust for future climate change than central females because southern females are already constructing nests close to their maximum possible depth. However, because climate is predicted to change more as latitude increases (Solomon et al., 2007), females from the Illinois population may actually be less able to

compensate for the effects of climate change because the predicted increase in temperature may exceed their capacity to construct deeper nests.

In conclusion, nest depth in painted turtles appears to be constrained by female body size because smaller females have shorter rear limbs with which to excavate the nest cavity. In addition, females from a warmer, southern site constructed deeper nests than females from a cooler, central site. Females from the southern site also appear to be constructing nests with depths closer to their maximum physical capability, which suggests that southern females may have less capacity to compensate for a warming climate by constructing deeper nests. However, because environmental temperatures are predicted to increase more at the central site, females from the Illinois population could be similarly threatened by climate change if the magnitude of temperature change exceeds their capacity for constructing deeper nests.

Acknowledgments.— This study was funded by the William Clark Graduate Student Award in Ecology and Evolutionary Biology (to J.M.R.) and NSF DEB-064932 (to F. Janzen). Thanks to T. Mitchell, D. Warner, and the 2010 and 2011 Turtle Camp Research Crews for help with data collection at the Illinois site; the U.S. Army Corps of Engineers for access to the Illinois site; A. Inslee and the staff at Bosque del Apache National Wildlife Refuge for hospitality at the New Mexico site; and to A. Bronikowski, P. Dixon, F. Janzen, C. Kelly, E. Takle, and H. Streby for helpful comments on the manuscript. This research was conducted in accordance with Institutional Animal Care and Use Committee protocols # 12-03-5570-J and 6-08-6583-J (Iowa State University), Scientific Collecting Permits 3430 (New

Mexico Department of Game and Fish) and NH10.0073 (Illinois Department of Natural Resources), and Special Use Permit 32576-OA022 (USFWS).

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Figure legends

Figure 1. Female painted turtle digging a nest. The turtle supports itself with its front limbs while using the rear limbs to excavate and shape the nest cavity (shown in cross-section). Illustration by Alyssa Hoffmeister.

Figure 2. Mean rear limb length regressed on plastron length for 93 painted turtles observed nesting in Illinois in 2010 (closed circles; $n = 83$) and New Mexico in 2010 and 2011 (open circles; $n = 10$). Because limb length was positively correlated with plastron length ($F_{1,91} = 10.92$; $P < 0.01$), plastron length was used as a measure of female body size in subsequent analyses.

Figure 3. Regressions of nest depth on clutch size (A) and female size (B) of painted turtles observed nesting in New Mexico and Illinois in 2010 and 2011. Slopes were not significantly different ($P = 0.42$).

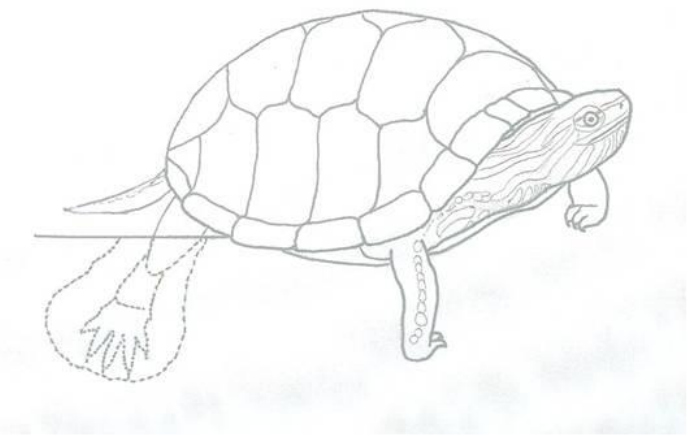


Figure 1

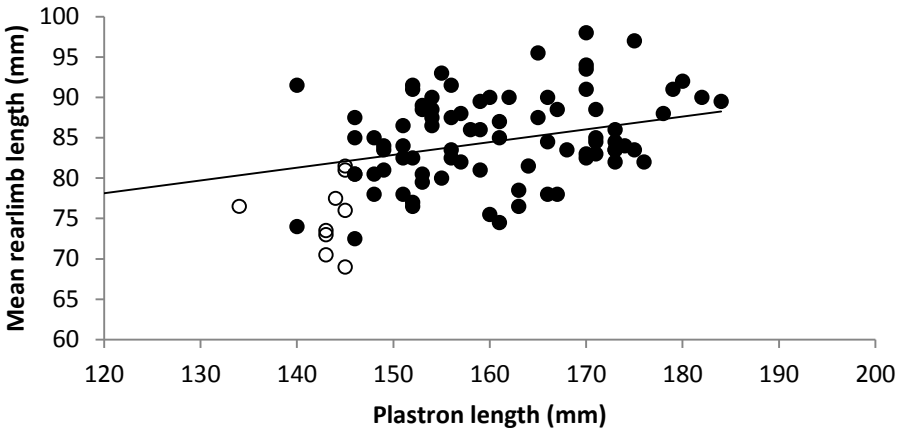
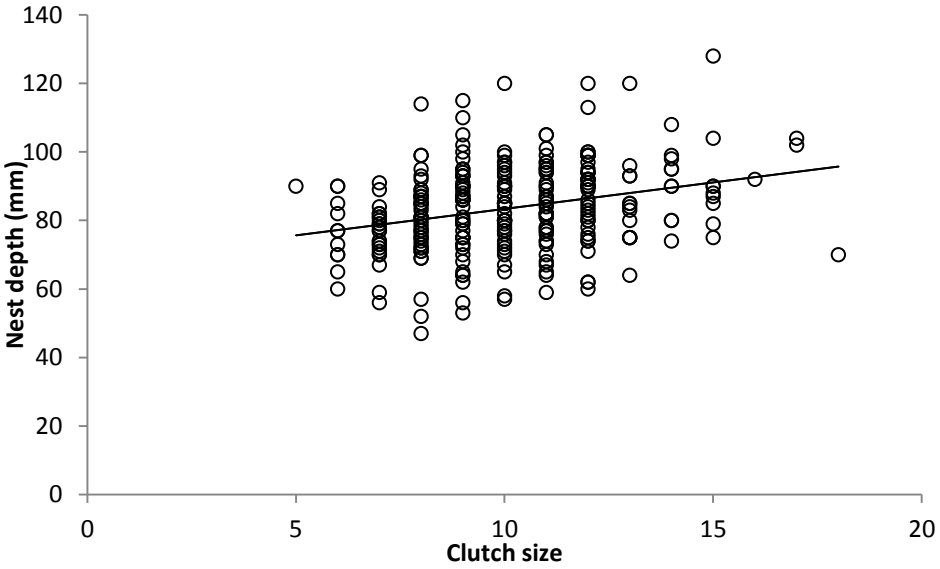
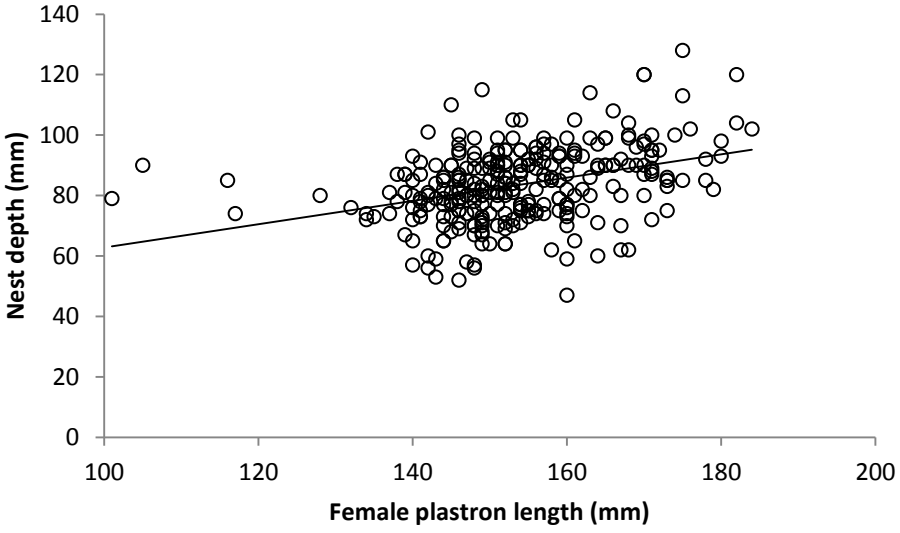

Mean rearlimb length (mm)
100
95
90
85
80
75
70
65
60
120
130
140
150
160
170
180
190
200
Plastron length (mm)

Figure 2


Nest depth (mm)
Clutch size
0
20
40
60
80
100
120
140
0
5
10
15
20

A


Nest depth (mm)
Female plastron length (mm)
0
20
40
60
80
100
120
140
100
120
140
160
180
200

B

Figure 3

CHAPTER 7. NEST DEPTH DOES NOT COMPENSATE FOR SEX RATIO SKEW CAUSED BY CLIMATE CHANGE IN TURTLES

A paper submitted for publication to *Oecologia*

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Abstract

Female ability to match nest characteristics with environmental conditions can influence offspring survival and quality, and may provide a mechanism by which organisms can track climate change. In organisms that construct subterranean nests, including many reptiles with temperature-dependent sex determination, the depth of the nest may affect incubation temperature and thus offspring sex ratio. Therefore, female adjustment of nest depth may be a mechanism by which skews in sex ratio caused by climate change could be prevented. We experimentally manipulated nest depth in 43 painted turtle nests and quantified the effects on incubation regime, offspring performance, and sex ratio. We found that nest depth affected the magnitude of daily temperature fluctuation, with deeper nests experiencing less daily variation, but mean nest temperature was not affected by depth. Within the biologically-relevant range of nest depths tested here, there was no difference in resultant offspring survival, size, or sex ratio. However, deeper nests produced hatchlings that were faster at righting themselves and swimming than hatchlings from shallower nests. We conclude that the adjustment in nest depth that would be required to affect sex ratio in this species is biologically unfeasible, and therefore female adjustment of nest depth is unlikely to compensate for climate change.

Introduction

Maternal choice of nest site and nest construction can have a large influence on offspring survival and quality (reviewed in Refsnider and Janzen 2010). For example, the amount of vegetative cover around a nest can reduce its visibility to predators (e.g., Martin and Roper 1988; Stokes and Boersma 1998), a nest's orientation may protect offspring from thermal stress (e.g., Walsberg and King 1978; Hartman and Oring 2003), and the temperature within a nest can affect offspring performance (e.g., Miller et al. 1987; Van Damme et al. 1992; Shine et al. 1997). Therefore, maternal ability to match nest construction to environmental and/or ecological conditions is an important determinant of a female's reproductive success. In the context of human-induced rapid environmental change, flexibility in nest-site choice and construction may be particularly critical in allowing females to shift nest characteristics to match changing environmental conditions and thereby continue to successfully reproduce.

A major category of human-induced rapid environmental change is global climate change, one result of which is a predicted increase in global temperatures of 1.1 – 6.4^{o}C by 2100 (Solomon et al. 2007). A temperature increase of this magnitude is likely to have catastrophic effects on species and ecosystems, but many of these outcomes are difficult to predict because they involve indirect effects of environmental changes on a multitude of organisms and occur via complex pathways. Some thermally-sensitive traits that are directly impacted by climate can have demographic consequences for populations. One example is temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos (Bull 1980). TSD occurs in many reptile groups as well as some fishes and invertebrates. In reptiles with TSD, the

temperature range within which the complement of offspring sex within a clutch shifts from all of one sex to all of the other sex is generally narrow, and is often less than 1^{o}C (Ewert et al. 1994). Consequently, population sex ratios are extraordinarily sensitive to temperature changes because a small shift in environmental temperature could dramatically alter offspring sex ratio (e.g., Schwanz et al. 2010). Human-induced climate change could severely impact reptiles with TSD by resulting in populations comprised of predominantly one sex (Janzen 1994; Mitchell et al. 2008).

Although adaptive changes in genotype frequencies in response to climate change have been observed in a few species (e.g., Bradshaw and Holzapfel 2001; Umina et al. 2005; Balanyá et al. 2006; Pen et al. 2010), the long generation times characteristic of reptiles with TSD and the low heritability of both embryonic thermal sensitivity and maternal nest-site selection (McGaugh et al. 2010; McGaugh et al. 2011) suggest that reptiles are unlikely to keep pace with rapid climate change through adaptive changes in gene frequency. Instead, if reptiles with TSD are to avoid skewed sex ratios produced by temperatures rising at unprecedented rates, behavioral phenotypic plasticity in nest-site choice may be the likely mechanism (Morjan 2003a; McGaugh et al. 2011; Refsnider and Janzen in press; but see McGaugh and Janzen 2011).

Nest depth may be one component of nest-site choice that females could vary in response to prevailing environmental conditions, and thereby match incubation regime with climate. Modeling studies suggest that nest depth affects both incubation temperature and the magnitude of temperature variation (Georges et al. 1994), and empirical data show that incubation temperature differs with depth in turtles (Roosenburg 1996), lizards (Shine and Harlow 1996), and crocodilians (Leslie and Spotila 2001). Indeed, altering nest depth as

global temperatures increase may be critical for some species; for example, tuatara (the sole representative of an ancient reptilian order) are predicted to produce all male offspring at current nest depths under maximum climate-warming scenarios (Mitchell et al. 2008).

Importantly, however, adjusting nest depth may not compensate for climate change in all cases. For example, in some species nest depth is not related to nest temperature (Warner and Andrews 2002), does not contribute to variation in sex ratio (Vogt and Bull 1982), or cannot be altered due to constraints on available nesting habitat (Mitchell et al. 2008). In other species, despite climatic differences among geographically-widespread populations, nest depths do not differ among populations (Doody et al. 2006a) or, when they do, such differences may be a function of female size (Morjan 2003b). Finally, even when nest depth changes in response to warming temperatures, such change may be insufficient to compensate for the magnitude of climatic warming (Telemeco et al. 2009).

The objectives of this study were twofold. First, we used a long-term dataset to determine whether mean nest depth of a wild population was correlated with annual climate. Second, we conducted a manipulative experiment to determine the effect of nest depth on incubation regime, offspring quality, and offspring sex ratio in a model species with TSD.

Materials and methods

Study species and site

We conducted this experiment using the western painted turtle, *Chrysemys picta bellii*. The painted turtle is widely dispersed across the United States and southern Canada, and the western subspecies occurs primarily west of the Mississippi River. This species inhabits a wide variety of aquatic habitats including rivers, lakes, and ponds. In early

summer, females emerge from wetlands to nest in open areas such as beaches and lawns. Our study site was a nesting beach on the northeastern side of a 1.5 ha island in the Mississippi River in Carroll County, Illinois, at the Thomson Causeway Recreation Area.

Data collection for the long-term study of nesting ecology is described in Schwanz et al. (2010). Briefly, we patrolled a nesting area in the South Potter's Marsh Campground hourly between 0600 h and 2100 h from mid-May through early July. We observed nesting turtles from a distance until they completed the nesting process, at which time we briefly captured females for individual identification before releasing them. We excavated nests within 24 hours of construction to assess clutch size, egg mass, and nest depth; we then re-covered nests with soil and left them to incubate in situ.

Data collection – long-term climate and nest depth trends

Nest depths have been recorded since 2000, and we used these data to determine the mean population nest depth in each year from 2000 – 2010, but focused on the grand mean population nest depth from 2004 – 2009 to determine our experimental treatments (see below). We used mean air temperature in May, acquired from the National Climate Data Center (www.ncdc.noaa.gov) for nearby Clinton, IA, as an indicator of climate in each year from 2000 – 2010.

Nest depth manipulation experiment

We conducted a nest-depth manipulation experiment on a subset of painted turtle nests constructed in the North Potter's Marsh Campground in June 2010. Nests were located and processed as described above. However, in this experiment we randomly assigned nests to one of three nest depth treatments: Shallow, Mean, or Deep. The Mean treatment was 8.7 cm, which was the grand mean nest depth for 1,126 nests constructed during 2004 – 2009.

The Shallow and Deep treatments were 6.7 and 10.7 cm respectively. These depths are two standard deviations from the grand mean, and were selected to represent biologically relevant values that, while relatively extreme compared to most nests, were not outside the range of physically possible nest depths for this population. To achieve the assigned nest depth treatment, we either added soil to the bottom of the nest chamber (to decrease nest depth) or excavated additional soil (to increase nest depth) before replacing the eggs. In cases where the entire clutch would no longer fit in a nest due to artificial decreasing of the nest depth, we increased the size of the egg chamber horizontally (rather than vertically) until all eggs would fit. In addition to manipulating nest depths, we inserted a temperature logger (iButton, Embedded Data Systems, Lawrenceburg, Kentucky, USA) amongst the eggs in the center of each nest. Loggers recorded nest temperatures hourly throughout incubation. Also, because canopy cover over nests affects incubation temperature (Morjan and Janzen 2003), we took a hemispherical photograph over each nest and quantified canopy cover using Gap Light Analysis software (as in Doody et al. 2006b). Finally, to minimize nest loss due to predation (which can range up to 95% of nests; Schwanz et al. 2010), we covered all nests with a 10-cm^2 piece of wire mesh staked at each corner.

We retrieved temperature loggers and all surviving hatchlings in September 2010 (after hatching but before nest emergence). For each nest, we considered the incubation period to start on day 0 (the day of oviposition) and continue through day 70. For reptiles with TSD, the thermosensitive period is generally the middle third of embryonic development (Wibbels et al. 1994). As we did not directly observe when hatching occurred in any nest, we considered days 16 – 45 to encompass the thermosensitive period during which sex differentiation occurs. We then calculated six parameters related to incubation

regime for each nest: minimum and maximum incubation temperatures (the lowest and highest temperature recorded during the 60-day incubation period), mean temperature throughout the incubation period (days 0 – 70) and the thermosensitive period (days 16 – 45), and the mean daily range (for each 24-hour period, highest recorded temperature – lowest recorded temperature) for the incubation period and the thermosensitive period.

Offspring quality and sex ratio

After retrieving hatchlings, we calculated the survival rate of each nest as the number of live hatchlings retrieved, divided by the known clutch size. We carefully cleaned and dried hatchlings, and recorded their plastrons with a color scanner to facilitate individual identification. We then weighed and measured (straight carapace length) all hatchlings and housed clutch-mates together in plastic deli cups containing moist soil over the winter at 4^{o}C in an incubator (Revco, Thermo Scientific, Asheville, North Carolina, USA). Starting in mid-March 2010, we gradually increased incubator temperature to 19^{o}C over a 2-week period and kept hatchlings at this temperature thereafter.

We conducted three performance tests on each hatchling: 1) righting time (the time it took for a hatchling placed on its back to right itself); 2) sprinting time (the time it took a hatchling to walk 0.5 m); and 3) swimming time (the time it took for a hatchling to swim 1.0 m). These performance tests simulated a hatchling's journey from the nest site to a wetland habitat, and then to suitable habitat within the wetland, following nest emergence in the spring. Therefore, we tested each hatchling in the order of righting time, immediately followed by sprinting time, and swimming time immediately following sprinting time. All hatchlings underwent two trials of three performance tests each, with the two trials separated by approximately two weeks. In all three performance tests, we timed a hatchling's latency

to begin moving (Latency), the total time taken to complete the test from initial placement of the hatchling until the test was completed (Total), and the time during which the hatchling was actively moving during completion of the test (Active, or Total – Latency). We recorded all times to the nearest second using a digital stopwatch, and censored all tests at 180 s. During the performance tests, researchers were blind to the depth treatment of the nest from which hatchlings were produced.

Upon completion of the performance trials, we sacrificed six hatchlings per clutch by a pericardial overdose of 0.5 mL of 1:1 sodium pentobarbital:water (the sex ratio of six sacrificed offspring per clutch reflects the sex ratio of the entire clutch; F. J. Janzen, unpublished). We assigned sex based on macroscopic examination of the gonads (Schwarzkopf and Brooks 1985), classifying individuals lacking oviducts and possessing short gonads as males (1) and those with complete oviducts and long gonads as females (0). After sexing, we preserved all specimens in 70% ethanol and released the remaining hatchlings at the collection site in May 2011.

Statistical analyses

We conducted all statistical analyses in SAS 9.2 (SAS Institute, Cary, North Carolina, USA). We first analyzed relationships between nest depth, year, and annual climate using general linear regression. We then compared each of the six incubation regime parameters among the three nest depth treatments using one-way analysis of covariance (ANCOVA) in the GLM Procedure with canopy cover as a covariate. We tested for differences among nest depth treatments in mean hatchling mass and carapace length using one-way ANCOVA in the MIXED Procedure with canopy cover and mean initial egg mass as covariates, and we tested for differences among these treatments in per cent hatching success using one-way

ANCOVA in the GLM Procedure with canopy cover as a covariate. Differences in nest sex ratios among nest depth treatments were compared using a chi-square goodness-of-fit test (Wilson and Hardy 2002). Because shade cover predicts sex ratio in natural nests at the study site, we also assessed whether shade cover continued to predict sex ratio of experimentally manipulated nests. This was done using logistic regression in the GENMOD procedure, with depth treatment and shade cover as independent predictors. Finally, we analyzed differences among treatments in median hatchling performance (i.e., Latency, Active, and Total median times for righting, sprinting, and swimming) using Kruskal-Wallis tests in the NPAR1WAY Procedure, and corrected the *P*-values for multiple tests ($m = 9$) using the Bonferroni adjustment.

Results

We analyzed the total depth of 2,371 unmanipulated painted turtle nests from 2000 – 2010. Mean May temperature did not significantly increase from 2000 to 2010 at the study site ($P = 0.96$; $R^2 = 0.0003$; Fig 1). Mean annual nest depth ranged from 8.48 to 9.11 cm (Table 1), and there was a slight but statistically significant positive correlation between nest depth and mean May temperature ($F_{1,2369} = 19.58$, $R^2 = 0.008$, $P < 0.0001$).

In 2010, we manipulated depths in 43 nests. Flooding of the Mississippi River in August caused data loggers to fail in six nests; therefore, we have incubation regime data for 11 Shallow, 13 Mean, and 13 Deep nests. Shade cover did not differ over nests assigned to the three depth treatments ($F_{2,41} = 0.22$, $P = 0.81$), nor was it a significant predictor of nest sex ratio ($\chi^2 = 3.3$; 1 df; $P = 0.07$). Minimum incubation temperature, maximum incubation temperature, mean incubation temperature, and mean thermosensitive period temperature did

not differ among nest depth treatments (all *P*-values > 0.10; Table 2). However, nest depth was inversely correlated with mean daily range during the incubation period ($F_{3,35}$ = 8.74, *P* = 0.0002) and the thermosensitive period ($F_{3,35}$ = 8.18, *P* = 0.0004; Fig 2); that is, deeper nests experienced less fluctuation in daily temperature than shallower nests.

As stated above, flooding of the Mississippi River during embryonic development caused the complete loss of 21 of 43 manipulated nests. The hatchlings in one additional nest were crushed by construction machinery shortly before we retrieved hatchlings from the nests, but because we were able to determine the sex of these hatchlings, they were included in the analysis of sex ratio differences among treatments. We retrieved 185 live hatchlings from the 21 surviving nests, and 165 of these hatchlings survived the overwintering period to be included in the performance tests.

Nest depth treatment did not influence per cent hatching success ($F_{3,35}$ = 1.27; *P* = 0.30), mean hatchling carapace length ($F_{2,135}$ = 0.90; *P* = 0.41), or offspring sex ratio (χ^2 = 6.3; 2 df; *P* = 0.10; Table 1), but hatchling mass decreased as nest depth increased ($F_{4,148}$ = 17.15; *r* = 0.56; *P* = 0.04). Hatchlings from the three nest depth treatments did not differ in any measure of median sprinting time (all *P*-values > 0.05). However, hatchlings from deeper nests had shorter Total righting times (χ^2 = 13.0; 2 df; *P* = 0.01), Active righting times (χ^2 = 16.6; 2 df; *P* = 0.002), Latency to swim times (χ^2 = 20.8; 2 df; *P* = 0.001), and Total swimming times (χ^2 = 13.6; 2 df; *P* = 0.01; Fig 3) than hatchlings from shallower nests.

Discussion

The strong influence of nest-site characteristics on offspring survival, quality, and phenotype illustrates the importance of maternal ability to match nest conditions and

construction with environmental characteristics. Matching nest-site characteristics and construction to prevailing environmental conditions may also be a behaviorally plastic mechanism by which organisms could track climate change, and thereby mitigate some negative impacts, such as skews in sex ratios in species with TSD. One nest-site characteristic that is potentially easily adjustable to match environmental conditions is nest depth, whereby females may simply construct deeper nests in warmer years. To determine the effect of nest depth on incubation regime, offspring quality, and offspring sex ratio, we experimentally manipulated nest depth within a biologically-relevant range in a model turtle species with TSD.

While spring temperatures (as indicated by mean May air temperature) at our Illinois study site did not increase over the course of the 11-year study, nests were deeper in years with warmer May temperatures. Also, *C. picta* nests in climatically warmer New Mexico are slightly deeper than *C. picta* nests at our northern site (Morjan 2003b). These results suggest some ability by female turtles to adjust nest depth based on prevailing environmental conditions. However, within the biologically-realistic range of nest depths tested here, the only difference in incubation regime between shallow and deep nests was in daily temperature variation. Mean, minimum, and maximum temperatures did not differ among nest depths. It is not surprising, then, that we observed no difference in the thermally-sensitive trait of sex ratio among our nest depth treatments. There were also no differences in hatchling survival or carapace length among depth treatments, although hatchling mass declined as nest depth increased. These results suggest that, while nest depths may be adjusted slightly under thermally divergent climate conditions, the adjustment is of insufficient magnitude to affect offspring sex ratio. Therefore, in order for nest depth to

compensate for potential sex ratio skews produced by climate change in this species, females at the Illinois site would have to construct nests at depths greater than two standard deviations from the current population mean. Because nest depth in turtles is believed to be constrained by female limb length (Tiwari and Bjorndal 2000; J. M. Refsnider, unpublished), selection for increased nest depth would likely necessitate a concomitant increase in female body size in order to construct deeper nests. The large shift in nest depth that would be required to affect incubation regimes sufficiently to alter sex ratios in this species seems unlikely to occur in response to climatic warming. Indeed, even in a reptile where nest depth is not constrained by female size and females did adjust nest depth to match climatic conditions, the adjustment was insufficient to prevent sex ratio skews (Telemeco et al. 2009).

Interestingly, we found that deeper (and thus less thermally variable) nests produced hatchlings that were faster at both righting themselves and swimming. In contrast, a common-garden experiment on the same species from five populations across a geographic range found that faster hatchlings were produced from nests that were more variable in daily temperature (Refsnider and Janzen in press). We expect that this difference is attributable to the latitudinal gradient in the common-garden experiment: in that study, female size increased with latitude, and the larger females from cooler climates constructed deeper, less variable nests; therefore, it may be the case that hatchlings whose mothers are from cooler climates (rather than hatchlings from less variable nests per se) are slower. Other studies have also found differing effects of fluctuating temperatures on performance of neonatal reptiles, even within the same population (e.g., Andrews et al. 2000; Les et al. 2007; Du and Feng 2008). For example, hatchling smooth softshell turtles (*Apalone mutica*) from an Iowa population were reported to swim faster as thermal variability during embryogenesis

increased (Ashmore and Janzen 2003), whereas a subsequent study of the same population found a less clear effect of such thermal variability on post-hatching swimming ability (Mullins and Janzen 2006). These diverse outcomes call into question the utility of generalizing about the effects of thermal variation experienced during embryonic development on the post-hatching performance of reptilian offspring. Indeed, results from our study suggest that the impacts of climatic warming on performance of hatching turtles are difficult to predict and likely will differ with latitude.

While the depth of turtle nests varies geographically and tracks May temperature at our Illinois study site, adjustment of nest depth is not likely to compensate for climate change. The amount by which nest depth would have to increase to affect sex ratio is greater than two standard deviations from the current population mean, and a shift of this magnitude is likely biologically unfeasible without a simultaneous substantial increase in female body size. Instead, shifts in other aspects of nest-site choice, such as selection of nest microhabitat, may be more realistic mechanisms of phenotypic plasticity by which turtles can match incubation conditions to a changing climate.

Acknowledgements

Thanks to T. Mitchell, H. Streby, J. Strickland, and D. Warner for help with data collection; A. Bronikowski, P. Dixon, C. Kelly, E. Takle, and H. Streby for helpful comments on the manuscript, and the U.S. Army Corps of Engineers for access to the study site. This research was conducted in accordance with Institutional Animal Care and Use Committee protocol # 12-03-5570-J (Iowa State University). This study was funded by Sigma Xi Grants-in-aid-of-

Research (to J.M.R.) and the National Science Foundation [DEB-064932 to F.J.J.]. The authors declare that they have no conflict of interest.

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Figure legends

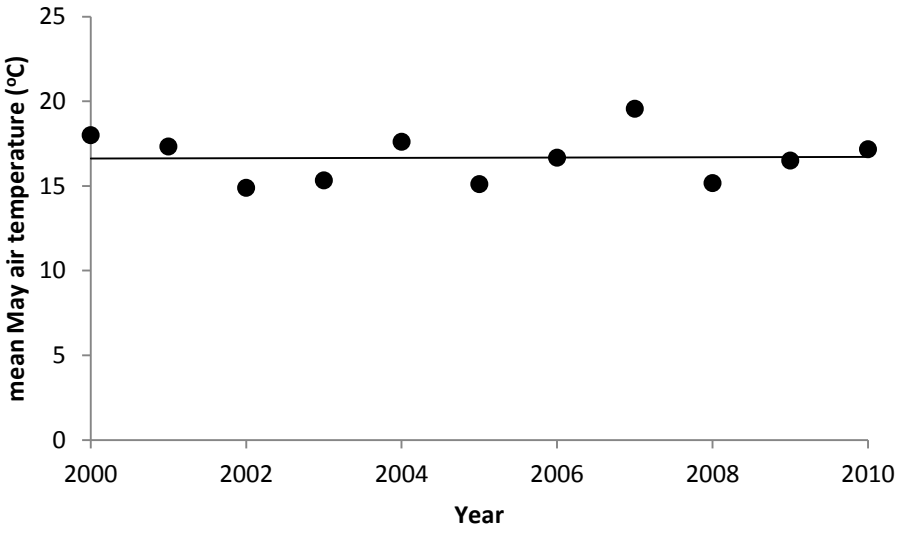
Figure 1. Mean air temperature in May (A) and mean depth (B) of painted turtle (*Chrysemys picta*) nests at Thomson Causeway Recreation Area, Carroll County, Illinois from 2000 to 2010. Nest depth was positively correlated with mean May temperature ($F_{1,2369} = 19.58$, $R^2 = 0.008$, $P < 0.0001$), but mean May temperature did not significantly increase from 2000 to 2010 at the study site ($R^2 = 0.0003$).

Figure 2. Mean daily temperature range throughout incubation (A) and during the thermosensitive period (B) in painted turtle (*Chrysemys picta*) nests of three different depth treatments at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010. Deeper nests experienced significantly less fluctuation in daily temperature than shallower nests.

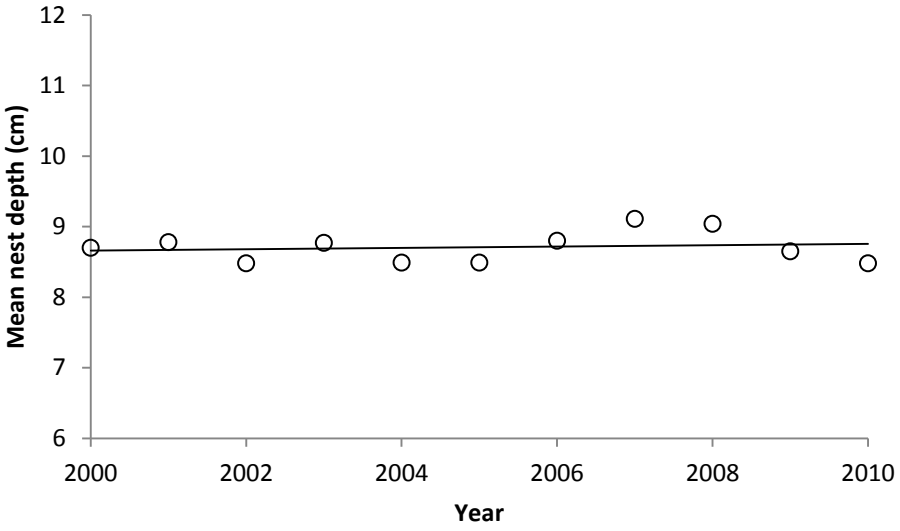
Figure 3. Nest medians for Total righting, Active righting, Total swim, and Latency to swim time of hatchling painted turtles (*Chrysemys picta*) from nests of three different depth treatments at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010. Deeper nests produced hatchlings that were faster to right themselves and to swim.

Table 1. Mean per cent hatching success, hatchling mass, hatchling carapace length (CL), sex ratio, and shade cover in manipulated painted turtle (*Chrysemys picta*) nests at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010. Values shown are means ± one standard deviation (number of hatchlings included in analysis). None of these variables differed significantly among nest depth treatments.

	Shallow (6.7 cm) (N = 11 nests)	**Mean** (8.7 cm) (N = 13 nests)	**Deep** (10.7 cm) (N = 13 nests)
% survival	57 ± 39 (75)	49 ± 41 (67)	26 ± 43 (43)
mass (g)	4.3 ± 0.6 (75)	4.1 ± 0.6 (67)	4.0 ± 0.6 (43)
CL (mm)	25.7 ± 1.5 (75)	25.2 ± 1.5 (67)	25.4 ± 1.4 (43)
% male	22 ± 25 (76)	8 ± 10 (67)	30 ± 21 (53)
% shade cover over nest	48.2 ± 6.6	48.0 ± 12.4	50.2 ± 10.2

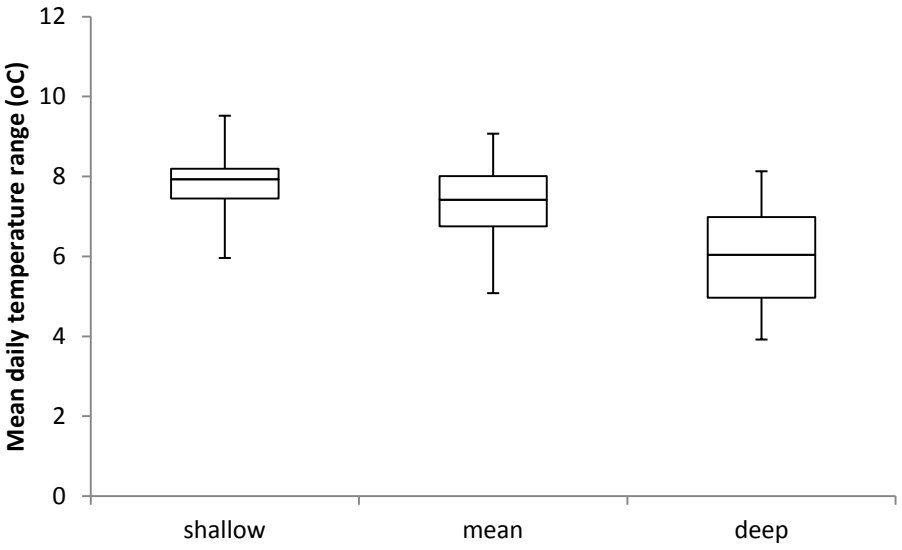

mean May air temperature (°C)
25
20
15
10
5
0
2000
2002
2004
2006
2008
2010
Year

A

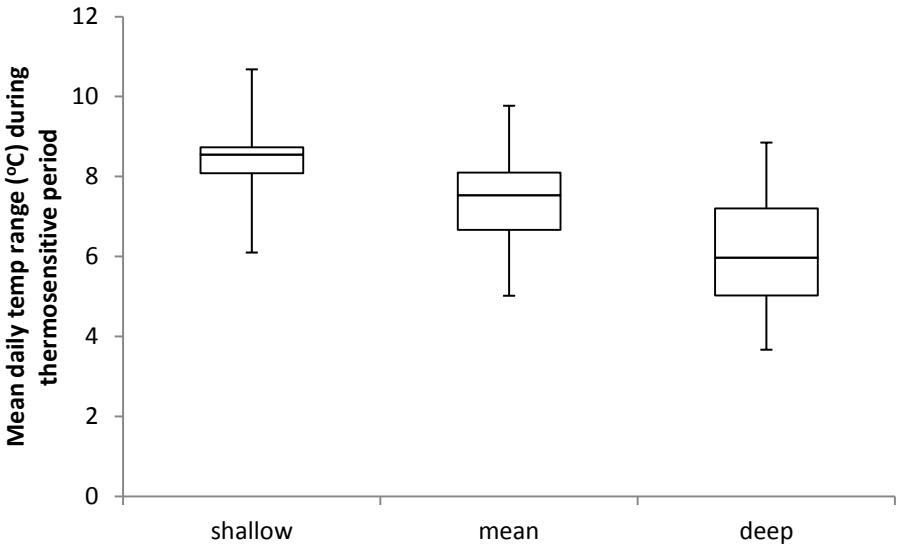

Mean nest depth (cm)
12
11
10
9
8
7
6
2000
2002
2004
2006
2008
2010
Year

B

Figure 1


Mean daily temperature range (oC)
12
10
8
6
4
2
0
shallow
mean
deep

A


Mean daily temp range (°C) during thermosensitive period
12
10
8
6
4
2
0
shallow
mean
deep

B

Figure 2

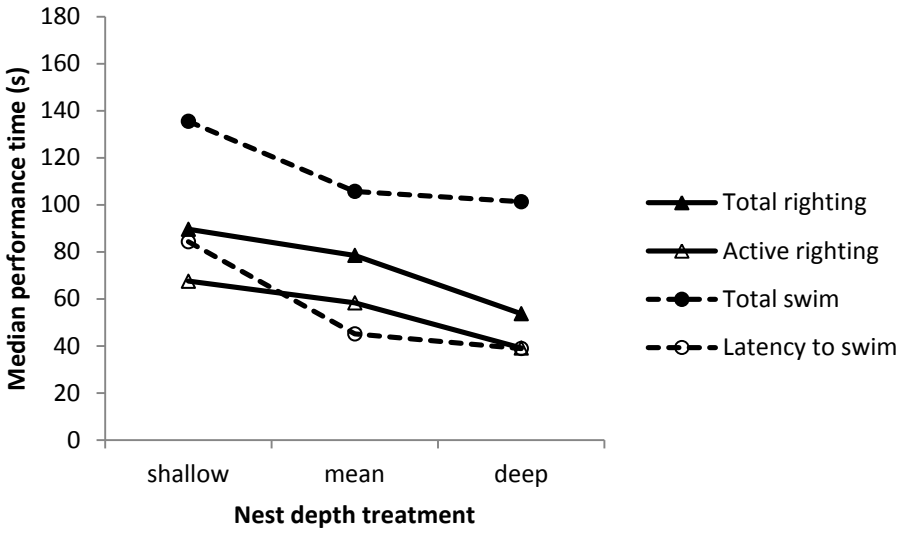

180
160
140
120
100
80
60
40
20
0
Median performance time (s)
shallow
mean
deep
Nest depth treatment
Total righting
Active righting
Total swim
Latency to swim

Figure 3

CHAPTER 8. GENERAL CONCLUSIONS

My dissertation provides a comprehensive test of the extent to which maternal nest-site choice behavior may allow reptiles with temperature-dependent sex determination to compensate for the potential skews in sex ratios caused by climate change. In the common-garden experiment described in Chapters 3 and 4, I demonstrated that female choice of shade cover over the nest is a behaviorally plastic characteristic that may allow reptiles with temperature-dependent sex determination to prevent sex ratio skews caused by climate change. Moreover, nests with greater mean daily fluctuation in incubation temperature produced faster hatchlings. This result suggests that if turtles are able to compensate for climate change via adjusting their choice of shade cover over the nest, such adjustment is likely to affect offspring performance.

Results of the study described in Chapter 5 indicate that selection of shade cover differs between populations, which I attribute to constraints on availability of shade cover as well as differences in parameters of incubation regime predicted by shade cover at each site. Importantly, for plasticity in choice of shade cover to be expressed, a range of shade cover options must be available in nesting areas from which females can select specific nest sites.

In Chapter 6, I show that nest depth in painted turtles appears to be constrained by female body size because smaller females have shorter rear limbs with which to excavate the nest cavity. Moreover, females from a southern population appear to be constructing nests with depths closer to their maximum physical capacity compared to females from a central population. My results suggest that southern females may have less capacity to compensate for a warming climate by constructing deeper nests than females from the center of the

species' range. Finally, in Chapter 7, I found that altering nest depth by two standard deviations from the population mean produced changes only in daily temperature fluctuation, and did not result in differences in sex ratio.

Overall, I found that choice of shade cover over the nest site is a behaviorally plastic trait, and although females can adjust selection of shade cover to compensate for environmental conditions, this adjustment affects the performance of offspring produced. Importantly, a range of shade cover options must be available to nesting turtles for this plasticity to be expressed. Nest depth is unlikely to compensate for the effects of climate change on sex ratio skews in turtles, as nest depth is constrained by female body size and the magnitude by which nest depth would have to shift to produce a difference in sex ratios is biologically unfeasible. In conclusion, maternal selection of shade cover over nest sites may be the component of nest-site choice most likely to compensate for potential effects of climate change on sex ratio skew in reptiles with temperature-dependent sex determination.

ACKNOWLEDGMENTS

I am grateful to many people for making this research successful. First and foremost, my advisor, Dr. Fred Janzen, and my committee members, Dr. Anne Bronikowski, Dr. Philip Dixon, Dr. Clint Kelly, and Dr. Eugene Takle provided advice and guidance throughout the entire dissertation process, with ideas for experimental design, analysis, and comments on manuscripts. Several fruitful side-projects came about as a result of their involvement and help, for which I am extremely grateful. Past and current members of the Janzen Lab and the Turtle Camp Research Crews were always available to provide insightful discussion, manual labor, and entertainment during long hours of fieldwork. In particular, I thank D. Warner and T. Mitchell for field assistance and great ideas, and B. Bodensteiner and J. Reneker for many hours spent racing hatchling turtles. Finally, I am indebted to Henry Streby for reading early drafts of all my manuscripts, extensive field assistance, and for supporting me throughout my graduate career.

This research was funded by the Iowa Academy of Sciences, Society for Integrative and Comparative Biology, Society for the Study of Amphibians and Reptiles and the Dean Metter Memorial Fund, Sigma Xi, Iowa State University's Department of Ecology, Evolution, and Organismal Biology Graduate Student Research Grants and the William Clark Student Award, and NSF DEB-0640932 (to Fred Janzen). Finally, this dissertation would not have been possible without Parafilm.